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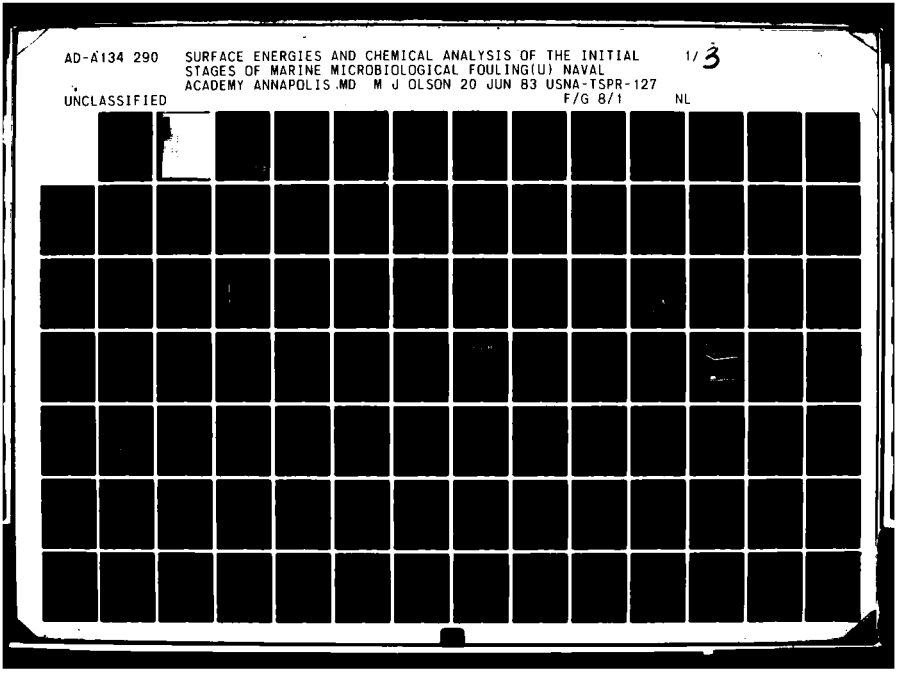

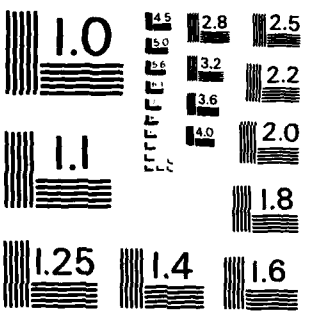

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"SURFACE ENERGIES AND CHEMICAL ANALYSIS OF THE INITIAL STAGES OF MARINE MICROBIOLOGICAL FOULING"

A Trident Scholar Project Report

by

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ABSTRACT

Marine biological fouling is a complex, successive process which can severely impair the performance of marine vessels. This process is expensive to the Navy due to the time and money used in fouling prevention and compensation. In this analysis, a series of three interrelated experiments were conducted which analyzed the initial stages of marine microbiological fouling in the Severn River estuary. In the first experiment, glass microscope slides were exposed to natural fouling organisms at "sampling stations" located along the length of the estuary. Slide and water samples were collected at set intervals, and a comparrison was made between the relative degree of fouling and individual water parameters experienced at each station. Results of the analysis showed low fouling to occur in waters which were relatively low in temperature and oxygen content, but high in salinity. The second experiment compared the surface energies of a set of substances to the degees of fouling and organism attachment they demonstrated. Surface energies were determined using a contact goniometer, samples were exposed to natural fouling organisms in a controlled environment, and degrees of fouling and organism retention were determined using dissection and scanning electron (SEM) microscopes, and a hydrous bacterial sprayer (HBS). Results showed definite surface energy ranges which would experience low degrees of fouling or organism retention. In the third experiment, an artificial sea water solution was created and organically enriched in order to analyze solutions which would support life and promote the transfer of fouling bacteria. It was discovered that each of the nutrient solutions would support life for an extended period of time, while straight artificial sea water and distilled water would only allow bacterial transfer for a limitied period of time. During each of the experiments, common or significant fouling organisms were observed and noted. One such observation in experiment two, discovered extraordinary organisms, or organism remnants, referred to as "sneep", which were significant due to their strength of adhesion on substrates, and their composition of primarily metals.

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I. INTRODUCTION

A. General Discussion of Marine Fouling

Marine fouling is a common, but complex, successive process which involves the settling, interaction, and subsequent accumulation of organic material, both living and non-living on a submerged solid surface (Corpe, 1977; Kelly, 1981). Zobell, in the 1930's and 1940's, discovered that fouling begins minutes after initial substrate submergence with the adsorption of a thin layer of organic material over the solid's exposed surface area. This layer, which is a product of the surrounding marine environment, provides a base for the adherence and growth of pioneer bacteria which begin to colonize the surface after a few hours of submergence. These bacterial colonies continue to grow and spread over the exposed area, becoming coated or encapsulated in envelopes of organic material which form polymeric bridges with the surface (Fischer, 1982). Within days after this initial colonization, microscopic plants and animals, such as diatoms and small protozoans, begin to adhere and grow upon the submerged "substrate". These organisms, combined with the already existing bacterial colonies, form a complex coating known as the "primary film", "bacterial fouling film", or "slime layer" (Horbiend and Frieberger, 1970). The latter of these names comes from the observation that this layer grows and develops until it reaches a state where it has a slimy or slippery feel (Haderlie, 1972; Kelly, 1981). The exact composition, rate of development, and

thickness of this layer depends largely upon surrounding environmental factors such as water temperature and chemistry, and the proximity of other surfaces supporting fouling organisms. (Kelly, 1981; Wilcox and O'Niel, 1971).

Once the microbial fouling film has been established, higher order marine fouling organisms such as animal larvae, large protozoans, and algae, begin to colonize the surface and continue growing over a period of several weeks. Fischer (1982) stated that in the 1950's approximately 2000 individual fouling organisms were known. This number has now been increased to include over 4000 organisms. Fischer also stated that the major organisms involved in the fouling succession were, chronologically, as follows:

(1) bacteria
(2) diatoms
(3) protozoans
(4) hydroids
(5) algae
(6) barnacles

Cologer (1979) extended this list to include tunicates (such as sea squirts) as indicators of the final fouling stage. This final stage or "climax" of the fouling succession occurs after the higher order fouling organisms living on the substrate have multiplied and become progressively larger. The climax stage is generally characterized by the presence of more visible fouling organisms, specifically sea weeds, bryozoans, large acorn barnacles, goose neck barnacles, and tunicates. This well developed layer of living matter completely covers the submerged portion of the sub-

strate. It continues to increase in thickness as existing organisms grow and reproduce upon already established layers and external organisms continue to attach.

B. Impact of Fouling on Marine Vessels.

The Navy spends millions of dollars annually attempting to prevent or limit the effects of biological fouling upon Naval Vessels. It has been conservatively estimated that ship hull fouling alone adds 150 million dollars to the annual Navy fuel budget (Fischer, Birnbaum, De Palma, Murooka, Dear, and Wood, 1975). Laster (1981) stated that this fuel penalty is the additional amount of fuel used to generate increased propulsion power necessary to overcome additional drag caused by fouling accumulation and hull paint deterioration. Additional costs, both in dollars and loss of ship operational capability, result from impaired performance and out-of-service time of ships, reduced service life, and loss of reliability of ocean surveillance equipment (Fischer, Birnbaum, De Palma, Murooka, Dear, and Wood, 1975). Fischer (1982) stated that recent studies have shown that biological fouling may induce drag increases on flow surfaces in excess of 10 percent. Since the frictional resistance of a ship at cruising speed constitutes 50 percent of its total flow resistance, and since a fuel consumption reduction of 1 percent would mean an annual savings of over 7 million dollars in the Naval fuel budget, the Navy is very interested in the fouling phenomenon. (Preiser, Cologer, Bohlander, 1979; Fischer, Birnbaum, De Palma,

Murooka, Dear, and Wood, 1975). Fouling of ship salt water intake piping can also adversely affect a ship's performance by creating additional water turbulence and by reducing water flow area and surface area necessary for proper heat excnange. A summary of the effects of biological fouling upon a ship operations as follows:

(1) increase drag (+10 %)
(2) loss of speed and maneuverability
(3) increased fuel consumption (to 50 %)
(4) decreased cruising range
(5) increased self-noise and acoustic signature
(6) acceleration of hull erosion
(7) loss of camouflage on submarines

(Fischer, Birnbaum, De Palma, Murooka, Dear, and Wood 1975)

Past anti-fouling research has focused on methods of cleaning or prevention in order to combat marine fouling. Cleaning of fouled surfaces by mechanical scraping or "scrubbing" is currently the most effective and popular cleaning method employed. This process is most efficiently and completely accomplished through the elaborate process of dry docking (Fischer, 1982). On the average, dry docking of Naval vessels occurs every three years, but shorter intervals may be required (Fischer, Birnbaum, De Palma, Murooka, Dear, and Wood, 1975). The high cost of this process and problems due to the toxicity of some cleaning agents used in dry dock cleaning have led to a push for longer time between dry dockings through the use of periodic "in situ" or "wet dock" cleanings (Fischer, 1982).

According to Fischer (1983) the most important areas for cleaning, as far as ship performance is concerned, are the first third of the ship's submerged hull, the sonar dome (if applicable), and the propeller. The propeller alone can account for 30 to 50 percent of the total fuel penalty experienced by the ship (Fischer, 1983). In one study involving a U.S. Navy Fast Frigate (FF), periodic cleaning of the ship's submerged surfaces resulted in what was termed as a "significant reduction" (14.6 %) in fuel costs (Laster, Preiser, Lehrer, 1981). Generally, the benefits of periodic cleanings to remove fouling organisms can be summarized as follows:

(1) Disruption of the natural biological succession of organisms that leads to gross fouling

(2) Effective and efficient removal of fouling when it is soft and less adherent to the surface

(3) Less likelihood of mechanical paint damage because of reduced brushing effort required

(4) Less deterioration of paint (biodegredation) which might be caused by the continued presence and growth of advanced stages of the fouling community

(5) Prevention of drag build-up with the consequent high level of ship performance and fuel saving

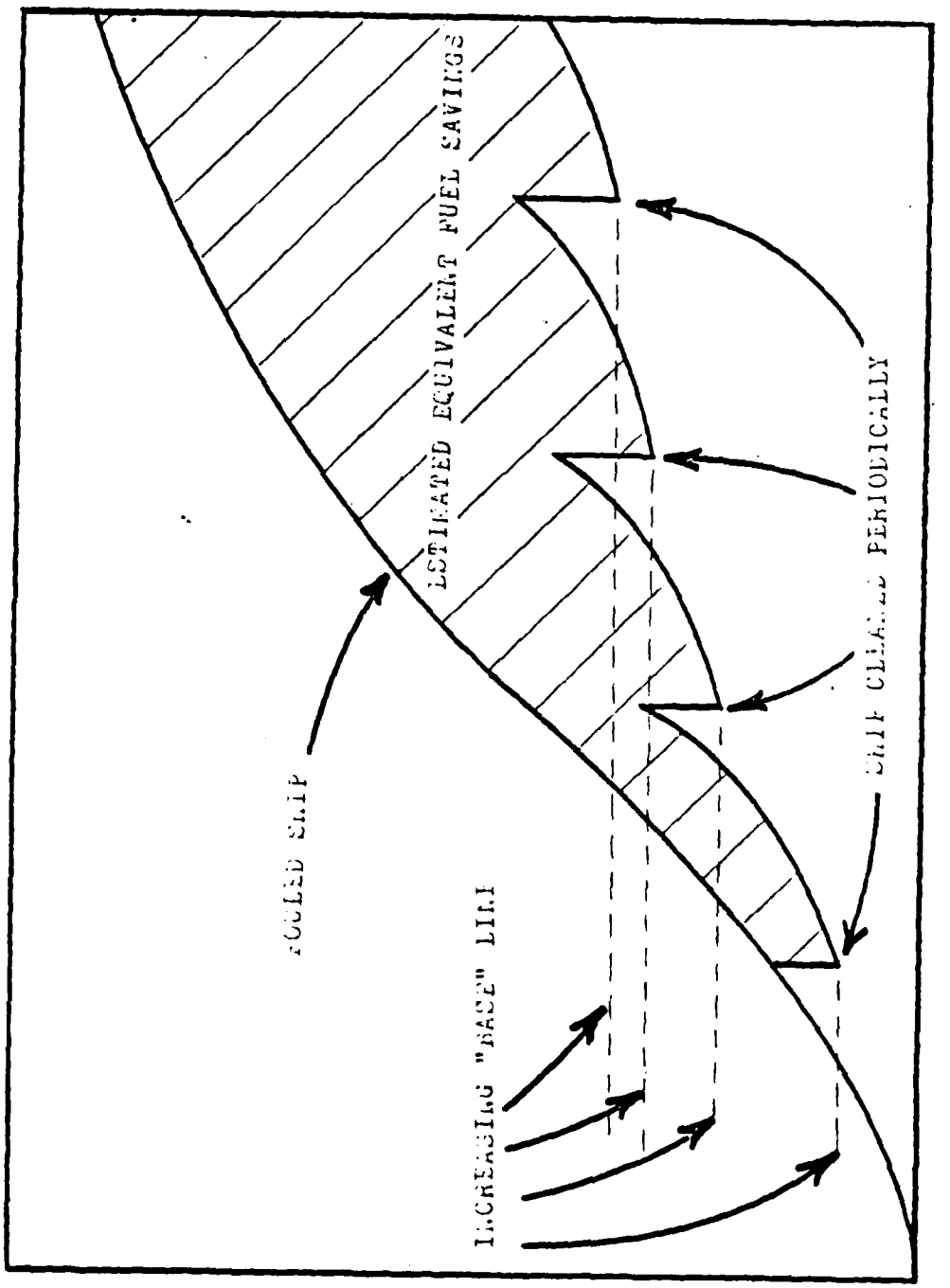
(Preiser, Cologer, Bohlander, 1979)

The primary disadvantage of cleaning is that it is neither a prevention, nor a cure to fouling, but simply a method to provide temporary relief from the adverse effects fouling may produce (Fischer, 1983). As shown in Figure (1), cleaning provides immediate, temporary relief to foul-

ing but, the hydrodynamics of past scrapings create roughness wnich won't allow the hull to get back to the original fuel consumption base line. With time and additional cleanings, this base line increases to a point of no return. Roughness also makes the null surface more attractive to fouling organisms, causing the re-fouling rate to increase as well (Fischer, 1983; Laster, 1981). Periodic cleanings can extend a ship's period between dry docks to up to 5 years, but as Laster (1981) points out in Figure (2), the amount of fuel saved by successive cleanings decreases rapidly. Cleaning by scrubbing can also be detrimental to a ship's hull surface. Improper use of scrubbing tools, the use of inappropriate brush materials, or inexperience of cleaning device operators can create blisters in the hull coatings, exposing bare metal and causing rapid corrosion of the hull (Fischer, 1983; Cologer, 1979).

FIGURE (1) - Conceptual Savings From Under Hull Cleaning

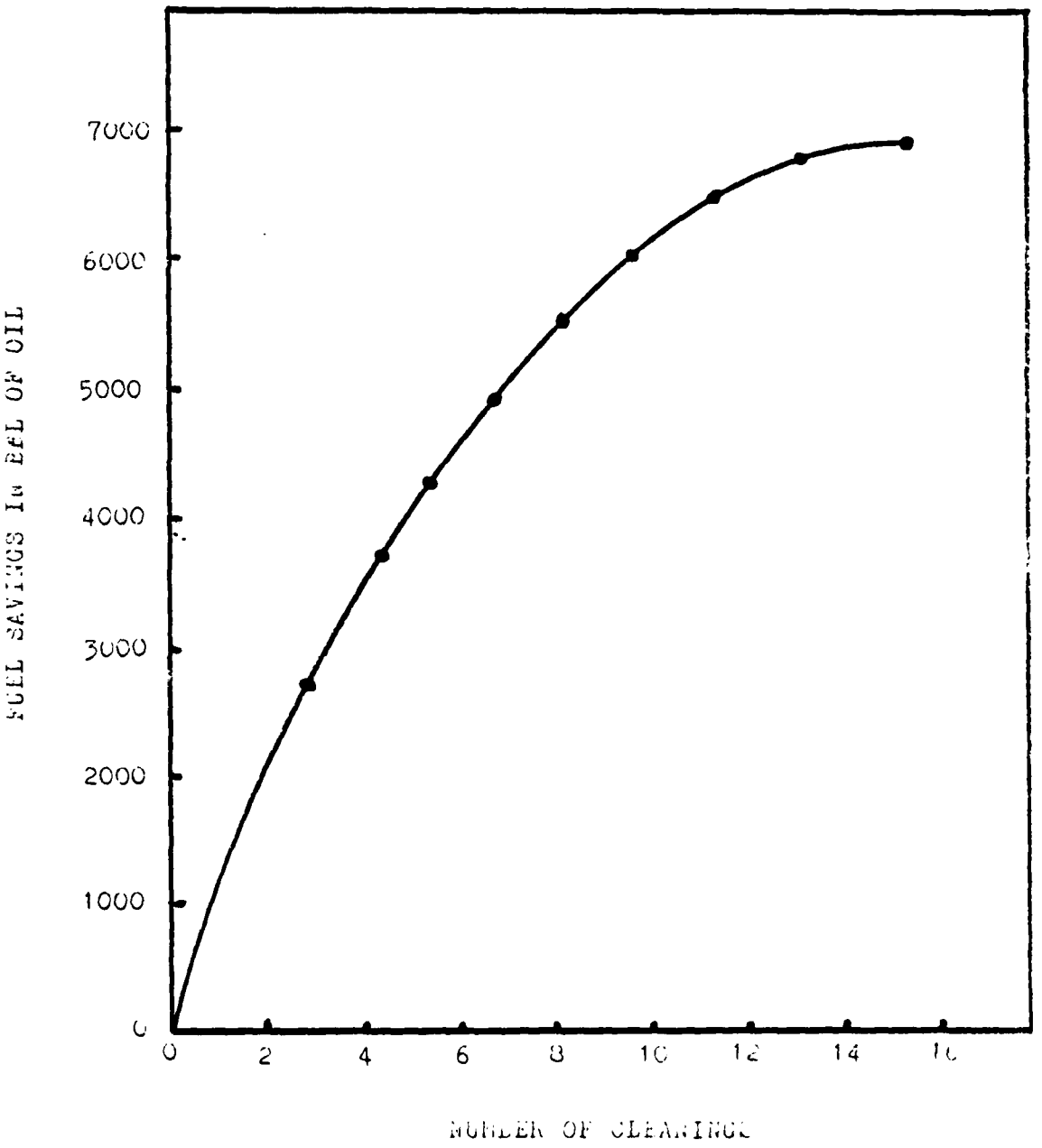
(From Laster, 1981)



FUEL PENALTY OR POWER INCREASE REQUIRED

TO MAINTAIN CONSTANT SPEED

FIGURE (2) - Annual Fuel Savings
Over 3-Year Operational Cycle



(From Lacter, 1981)

Past methods to prevent marine fouling have concentrated primarily on the development of toxic anti-fouling paints. These coatings attempt to prevent the initiation of the biologic fouling succession by making the surface inhospitable (poisonous) for the growth of pioneer fouling animals and plants. The basic idea is to create a pesticide with low mammalian toxicity, but high target toxicity, primarily aimed at encrusting marine organisms (Fischer, 1983). Originally, the toxic chemicals used in these coatings were mercury, oxides of lead and copper, and even arsenic (Fischer,1982). These chemicals were mixed into an organic matrix material in order to allow their gradual releases, or dissemination into the surrounding environment. As Fischer (1982) stated:

> Traditional antifouling coating systems are mixtures containing sufficient water-soluble resins, pigments, metal salts, and inert fillers for direct contact to occur between the particles within the paint film; as one particle dissolves, another in contact with it is exposed to hydrolytic action. This process, called leaching, varies with factors such as coating age, water velocity, temperature, salinity, and primary film layer.

Using the traditional process, the toxic chemicals were not actually bonded to the matrix material. This physical admixing often caused a complete leaching out of the toxic materials within 18 months (Fischer, 1983). Current antifouling paints no longer use mercury or arsenic, due to their toxicity, but still incorporate oxides of copper and lead, along with a new series of toxins known as "organometallic polymers" or OMP's (Kelly, 1981; Fischer, 1982).

These substances, first developed in the early seventies, chemically bind a pesticide to a polymer backbone, providing a controlled release (dosage) of the pesticide over a longer period of time than possible with previous coatings. The most common metals used in OMP's are tin and lead, whose compounds account for over 300 of the materials and combinations of materials currently used in antifoulants (Fischer, 1982). The majority of these paints are still in the developmental stages, however, and traditional copper-based paints remain the most popular coating choice. Copper oxide coatings generally do not last as long as OMP's, but they are less expensive and have a more stable history. One defective batch of OMP coatings lost all its antifouling toxin and fouled within six months, while others have lasted several years (Fischer, 1983). Listed in Table (1) are the most common antifouling paints available today, along with their cost per gallon, and average lifespan in months. The main advantage to toxic antifouling paints is that, like scrubbing, they are effective at providing temporary relief from the problem of fouling. In some cases copper oxide paints have maintained a 90 percent barnacle resistance for as long as 4 years, while maintaining a 90 percent total fouling resistance for up to one year (Fischer, 1982). New OMP paints may extend this life span significantly, making the desired Navy goal of 5 to 7 years between hull repainting possible (Fischer, 1983).

TABLE (1) - Common Antifouling Paints

Paint	Average Price ($/Gal.)	Ave. Life (Months) *
Copper Oxide	32	10 to 18
Organotin Polymer	31 to 34	5 to 42
Organolead polymer	50+	up to 50
Tributyltin Polymer	50+	up to 50
Tributyltin Oxide	50+	up to 52
Tributyltin Fluoride	50+	up to 51
Tributyltin Acetate	50+	up to 51

(Fischer, 1982, 1983)

* Service life may vary with coating thickness, ship deployment, and surrounding environment.

The relatively inexpensive copper paints normally only account for 0.4 to 0.8 percent of a ships total operational cost, while greatly reducing its fuel consumption during the months shortly after painting. Paints, however, have their limitations.

The main limitation to paints is that, since they are not permanent and must therefore be periodically replaced, a large cost is produced, even through the use of the relatively inexpensive copper paints. It has been estimated that the Navy prepares and paints 10 million square feet of ship hull surface with antifoulants annually (Fischer, Birnbaum, De Palma, Murooka, Dear, and Wood 1975). The labor cost alone of this procedure is more than 15 million dollars. A single aircraft carrier can use as much as 10,000 kg of antifouling paint in a single coating, which often must be replaced in as little as 8 months (Fischer, 1982).

Limitations to the use of antifouling paints also occur due to the toxicity of these coatings. The leaching out of poisonous chemicals into waters surrounding a vessel can have adverse effects upon the existing environment. In closed harbors, rivers, and other locations where tidal flushing may be limited and many ships may be present, the environmental impact of toxins from antifouling paints may be substantial. Because of their toxicity, antifoulants are classified as pesticides and therefore must be registered under the Federal Insecticide, Fungicide, and Rodenticide Act (Fischer, 1983). Laws of a restrictive nature under the

"Pesticide Programs" section of the Code of Federal Regulations also make introduction and testing of new agents difficult and expensive. Current registration fees range from 50 to 150 thousand dollars, while antifoulant testing can cost as much as 3 million dollars (Fischer, 1982).

C. Recent Aspects in Fouling Prevention.

Since neither cleaning nor toxic paints provide a total solution to the problem of biological fouling, researchers have begun to consider other aspects of the fouling succession which may lead to better preventive methods. This study concerns one of these aspects; that of microbiological fouling.

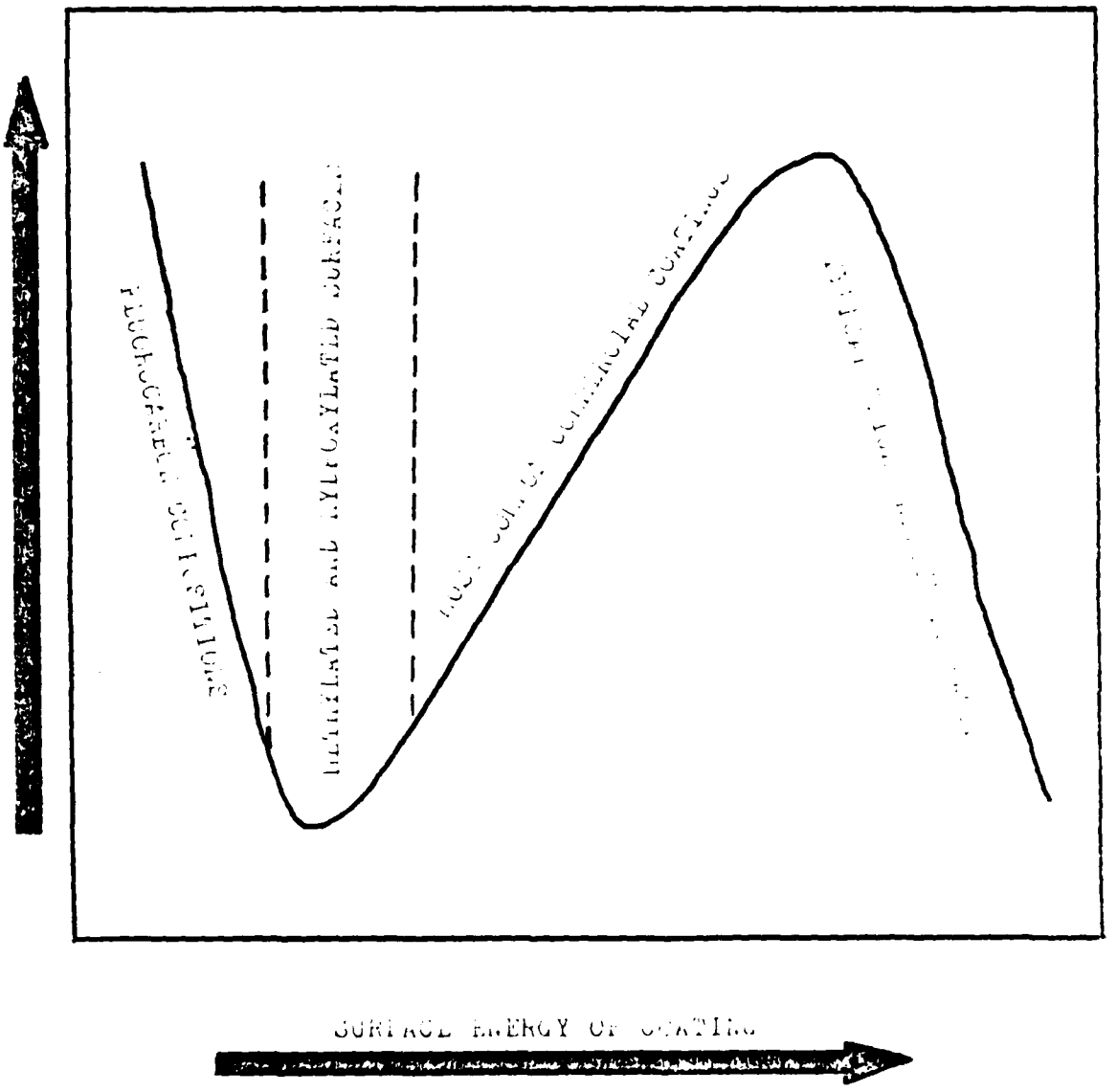
Microbiological fouling, or microfouling, consists of those stages of the fouling succession which lead to the formation of the complex bacterial "slime" layer (Kelly, 1981). It was initially believed that this layer was the first stage of the fouling succession, but the work of Zobell in the 1940's and more recent work by Loeb and Neihof in the 1970's demonstrated that the actual initial stage is that of an organic film which forms on a substrate within minutes after initial submergence (Kelly, 1981). This layer, which is composed of organic materials such as sugars, amino acids, urea, and fatty acids, originates as the end-products of bacterial decay, excretions by marine organisms, dissolutions from plant life, and other similar processes (Kelly, 1981; Riley, 1963).

Loeb and Neihof (1975, 1977) determined that the initial organic film will grow to a thickness of about 20 A in a few hours and then continue to grow until a leveling off period occurring after approximately 20 hours. This layer "conditions" the surface of the substrate, meaning that it changes the surface to enhance additional attachment of microbial fouling organisms. Baier (1977) along with Goupil and De Palma (1973) discovered that this conditioning had an effect upon the rate and degree of fouling a substrate experienced. Characteristics such as texture, chemistry, electromagnetic charge, and to a large degree, the "critical surface tension" or "wettability" of the substrate influenced the initial adhesion of fouling organisms to that substance (Kelly, 1981). This latter characteristic, which is also referred to as a substance's "surface energy", is the work required to bring a molecule from the substance's interior to its surface. As illustrated in Figure (3), Baier (1977) demonstrated that there was a relationship, although not direct, between a substance's surface energy and the degree of biological fouling it experienced. This correlation was found to occur in a number of solutions, including sea water and human blood. Baier also stated that the surface energy range which experienced the lowest degree of fouling was that occurring between 20 and 30 dynes/cm. Dexter (1977) further narrowed this "minimal fouling" range down to between 22 and 24 dynes/cm. As Fischer (1982) pointed out, however, this did not mean that substances

whose surface energies lie within this range would not foul, but rather that they would foul at a rate relatively slower than substances whose surface energies occur significantly to either side of the range.

Kelly (1981) stated that as molecules are adsorbed onto a surface, they change from a three-dimensional to a two-dimensional form which modifies their activity. This modification of the substrate's surface allows pioneer bacteria to firmly attach by exuding an encapsulating mucopolysaccharide "glue" which causes the beginning of the "secondary film" (Kelly, 1981; Baier, 1977). This layer forms within hours after initial submergence as bacterial colonies become well established on the substrate's surface. Dempsey (1981) showed these bacteria to be as much as eighty-five to ninety percent gram negative. The first bacteria to attach are a rod-shaped type known as "Pseudomonads". These have been further identified to be primarily species of Pseudomonas, Flavobacterium, and Achromobacter (Kelly, 1981; Corpe, 1973; Corpe and winters, 1972; O'Niel and Wilcox, 1971). Within the first day or two after submergence, often as many as forty or fifty species of stalked, budding, or filamentous bacteria belonging to Caulobacter, Hyphomicrobium, and Soprospua begin to appear on the submerged surface as well (Kelly, 1981; Dempsey, 1981; Marshall, 1971; Zobell and Allen, 1935).

FIGURE (5) - Surface Energy vs. Degree of Fouling



(From Baier, 19[illegible])

Bacteria colonize a substrate in two basic stages referred to as "reversible sorption" and "irreversible sorption" (Marshall, 1971). The first of these stages is characterized by the bacteria being weakly held to a surface by physical attractive forces such as Van Der Waals forces of mass attraction and electrostatic forces caused by ionic groups interacting on or around the approaching particles and the substrate surface (Kelly, 1981; Dempsey, 1981). During this stage the bacteria reach a state of equilibrium between these attractive forces and the repulsive forces that surround them. This state of equilibrium still allows them to exhibit Brownian molecular motion, and the weak bonding makes them easily washed from the substrate surface (Marshall, 1971). After a few hours of contact with a substrate, however, the bacteria begin forming more secure bonds with the surface. This marks the beginning of irreversible sorption.

Irreversible sorption occurs once the bacteria, especially the rod-shaped bacillus types, exude the acid mucopolysaccharides mentioned earlier. These chemicals create extracellular bridging materials which firmly attach the bacteria to the substrate surface and make neither Brownian motion nor removal by plain water washing possible (Kelly, 1981; Corpe, 1970). Once settled onto a substrate surface, the bacteria grow and reproduce by binary fission, with each half growing to an average size of one to two microns and then dividing again (Kelly, 1981). As mentioned previously,

these dividing bacteria spread and form extensive colonies, which when combined with other organisms that also may have colonized the substrate such as algae, protozoans, and diatoms, form the secondary film or "slime" layer.

Zobell (1939) and Corpe (1977) gave several functions that the "slime" layer possessed. Both believed that the film initially provided a favorable base for the adhesion and settlement of animal larvae and algal cells, and later provided a rich food source for the growth and development of these organisms, but Zobell extended this to include:

(1) discoloring bright or glazed surfaces

(2) increasing the film-surface alkalinity, thus favoring the deposition of calcareous sediments (such as those used to create barnacle shells)

(3) influencing the electromagnetic force (emf) potential of the surface to make it more favorable for biological growth

(4) increasing the existing amount of plant nutrients at the expense of organic matter

(Kelly, 1981).

Both researchers agreed that the bacterial slime layer provides a basis for the subsequent attachment and growth of higher organisms in the fouling succession. This bacterial stage, however, is heavily based upon the "conditioning" microfouling film of organic material established hours after initial submergence. Therefore, if either of these two initial stages could be reduced or controlled, latter stages of fouling could be greatly deterred or diminished.

II Objectives.

A. General

The major objectives of this project were to study elements of marine microbiological fouling, specifically, the growth rates, adhesive strengths, and chemical factors affecting the fouling organisms in the Severn River estuary system. These characteristics were chosen because of their importance, both in an analysis of the fouling succession, and as a basis for antifouling research. Experiments to evaluate each of these areas were individually performed during a nine month period between August, 1982 and April, 1983. All tests were done with equipment and materials of the United States Naval Academy or the David W. Taylor Naval Ship Research and Development Center (NSRDC) Annapolis, Maryland, with the exception of the glow discharge plasma cleaner used in experiment two, which was borrowed from the Environmental Center of Anne Arundel Community College, Arnold Maryland.

B. First Experiment Objectives.

The objectives of the first experiment were to:

(1) Establish "fouling stations" along the length of the Severn River Estuary.

(2) Expose glass microscope slide samples to naturally existing fouling organisms at each station.

(3) Monitor the water characteristics along with fouling organism growth at each station over a period of time.

(4) Analyze and compare measured water variables to the degree of fouling experienced at each station.

C. Second Experiment Objectives.

The objectives of the second experiment were to:

(1) Obtain a set of substrate samples of varying surface energies.

(2) Experimentally measure and determine accurate surface energy values for each substrate sample.

(3) In a controlled environment, expose substrates to natural fouling organisms.

(4) Experimentally evaluate the relative degree of fouling and tenacity of organism attachment for each substrate.

(5) Compare substrate fouling characteristics of substrates based on their established surface energies.

D. Third Experiment Objectives.

The objectives of the third experiment were to:

(1) Obtain samples of pioneer fouling bacteria.

(2) Create an inorganic artificial sea water solution.

(3) Modify and organically enrich the artificial sea water to allow bacterial transfer.

(4) Analyze solution(s) which allow bacterial transfer in an attempt to isolate a common agent which is necessary for initial bacterial fouling.

(5) Expose small sections of microscope slides to samples of purified fouling bacteria, removing each at set time intervals.

(6) Analyze and photograph the initial stages of fouling, in terms of bacterial colonization.

III Materials and Methods

A. General

The first of the series of analytic experiments involved the analysis of fouling in the Severn River using microscope slides suspended at stations along the estuary's length. They were carried out during the period between 19 October and 2 November, 1982. Experiment two, involving the exposure of substrates of different "surface energies" to naturally occurring fouling organisms, was conducted during the period from 17 August, 1982 until 4 March, 1983. The third series of experiments, designed to explore the components of the initial organic film layer produced by the surrounding marine environment, were conducted between 14 December, 1982 and 15 April, 1983. During each of these experiments, photographs, notes, or drawings were made of the equipment used and the procedures performed.

B. Experiment One - Analysis of fouling in the Severn River Estuary, Anne Arundel County, Maryland.

1. Sampling Boxes

Standard 10.6 cm by 8.6 cm microscope slide boxes were used as slide holding cases. These boxes were prepared by cutting a 8.6 cm by 6.6 cm "window" into the front and back face of each box in order to allow free flow of sea water through the box. Two 0.33 cm holes were drilled for the attachment of a wire hanging clip. A twelve foot section of .35 cm nylon cord with a small polystyrene float was secured

to each hanging clip, and twelve 7.5 cm by 2.5 cm plain, precleaned microscope slides were loaded into each box. To prevent accidental opening, each box was enclosed by two rubber bands wrapped horizontally around the front and back faces. Shown in Figure (4) is a representative prepared slide box.

2. Sampling Stations

Seven sampling stations were established on both private and public land along the length of the estuary. At each station two, or in some cases one slide box was attached to an existing dock or pier. Enough slack line was made available to compensate for daily tidal fluctuations, while the polystyrene float maintained the box at a constant depth of 1/2 meter below the water. A suspended slide box is illustrated in Figure (5). Stations were alphabetically sequenced beginning at the mouth of the Severn, while boxes at each station being numerically designated in the same manner. A map of the Severn River including station locations, names, and numeric designations is shown in Figure (6).

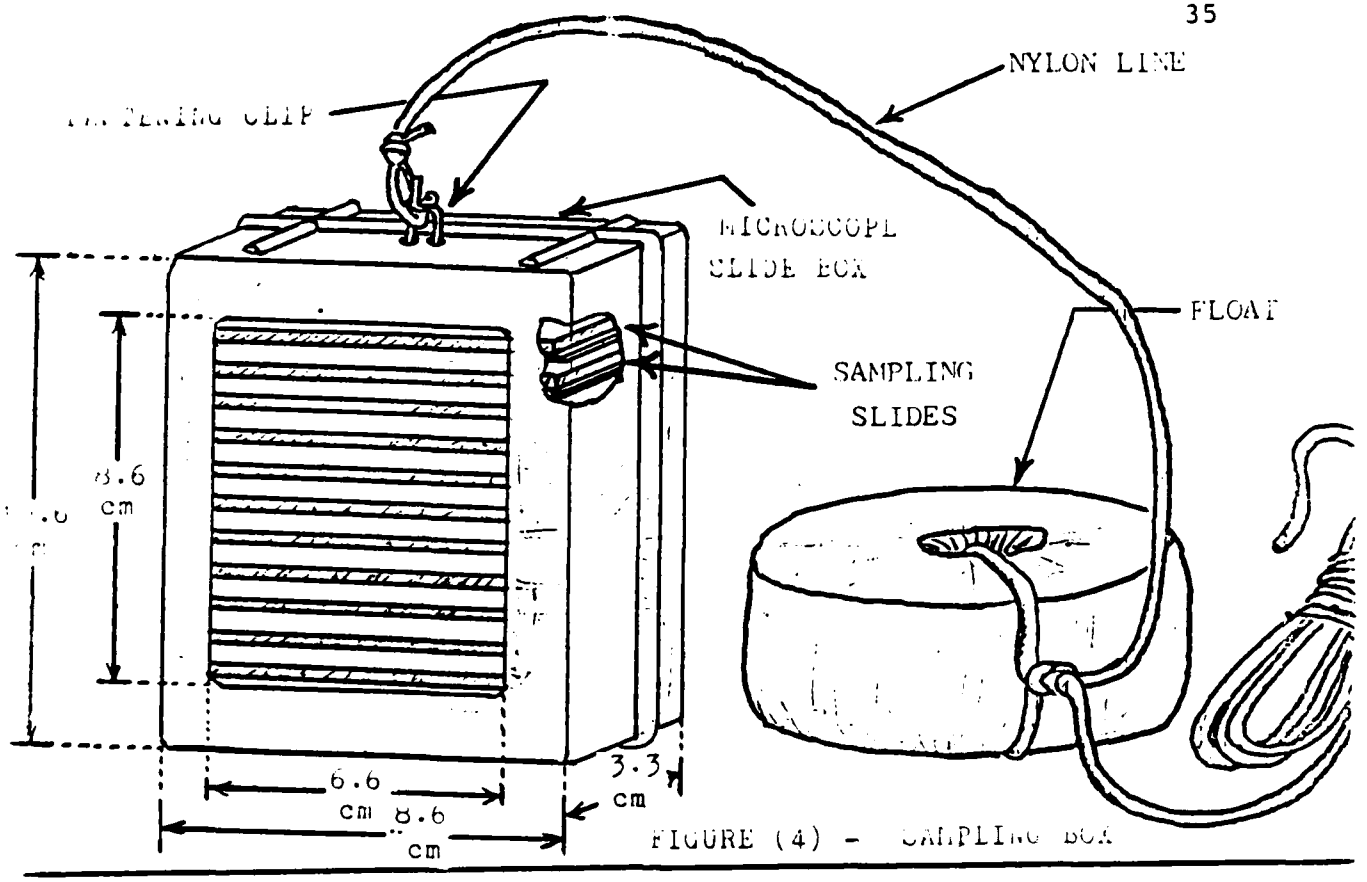


FIGURE (4) - SAMPLING BOX

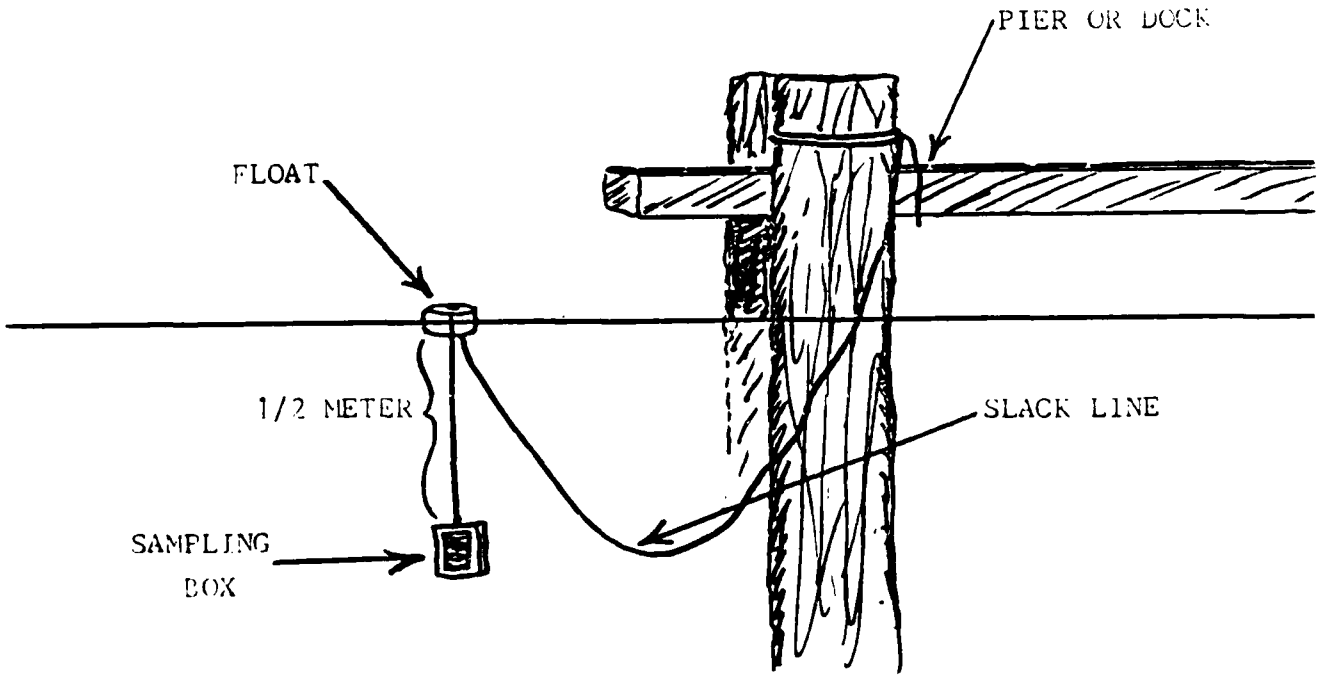
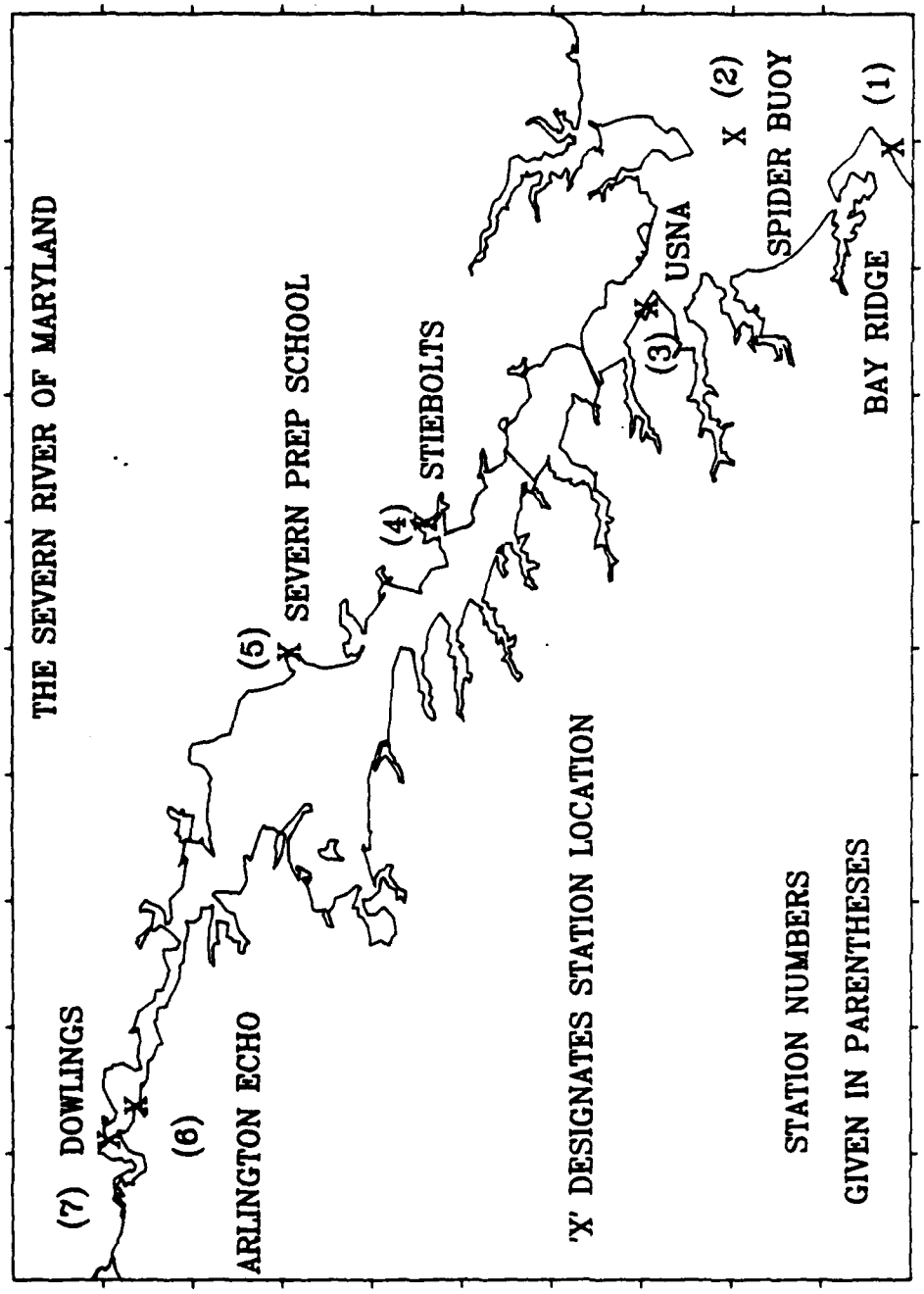


FIGURE (5) - SLIDE BOX SUSPENSION

FIGURE (6) - THE SEVERN RIVER



3. Sampling Procedures

At the time of initial submergence, water samples were taken at each station and conductivity, salinity, temperature, and oxygen content were measured. These water variables, along with microscope slides samples, were taken at each station after submergence periods of one, three, seven, ten, and fourteen days. Before collection visits to stations were made, the water analysis equipment to be used was calibrated and checked. The two pieces of equipment used were a conductivity, salinity, and temperature (CST) meter and an Oxygen/Temperature (OT) meter. The first of these was calibrated on a daily basis by placing a 47.58 ohm resistor into the ring of the dry sampling probe and "zeroing" the device using the calibration set screw. Once calibration had been done, the meter was not adjusted further that day.

The oxygen Temperature (OT) meter was calibrated once at the beginning of the sampling period using a standard oxygen solution. Due to the complexity of this calibration, it was only done at the beginning of each sampling period. The meter was re-charged daily, while not in use, to ensure proper voltage for accurate readings.

Once equipment was calibrated and fully charged, stations were visited for data collection. Samples of the water surrounding each slide box were taken using a common two gallon bucket into which the probes from the CST and OT meters were placed. The OT meter had a built-in stirrer which was engaged as soon as the probes were in the water sample. Water samples were allowed to settle for approximately one minute before readings of temperature, conductivity, salinity, and oxygen content were taken. Each data value was logged along with it's appropriate station number and time of collection. After water variable readings had been taken, the sample was discarded.

On each collection day a non-modified ("window-less") microscope box was selected and marked as that day's "transfer box". This box was filled with Severn River water to prevent dehydration of the samples to be collected that day. At each station, a representative slide was removed at random from that station's suspended box and placed in the transfer box. To avoid confusion, slides were numerically set in the built-in numbered rack of the transfer box, with the number of the parent slide box corresponding to the slot number in the rack. This procedure was repeated at each station until the transfer box contained all the slide samples for that collection day.

4. Processing Procedures

Upon completion of sample collection, the transfer box was inverted and drained of its water. The slides were then sequentially removed from the box, rinsed with tap water to dislodge any loose material, suspended for two minutes in a crystal violet solution, gently rinsed again with tap water, and sequentially placed back into the transfer box. The transfer box was then inverted over a paper towel as slides were dried for twelve hours. Once slides were completely dry, they were numbered using a diamond scratching tool.

5. Analysis Procedures

Microscope slide samples were viewed with a Leitz wetzler microscopic camera system, which allowed microscopic analysis and direct photomicrography of the subject viewed. Analysis was done at lens magnifications of 35, 60, 100, and 400 power. Slides were analyzed on the basis of amount of fouling growth and types of organisms present, with special attention being placed on any growths, colonies, or organisms which were abundant or conspicuously common. Photographs of slides were taken to show an overview of existing fouling conditions or of specific items which were of interest for later analysis.

Slides were also relatively ranked based on the amount of fouling they exhibited for that day. The slides from each transfer box were placed on a sheet of white paper, and the slide with the least amount of fouling was designated a value of one, while the slide with the greatest relative

value of one, while the slide with the greatest relative amount of fouling was assigned a value of 20. Each remaining slide was assigned a number between one and 20 based on its individual relative degree of fouling.

All data values obtained from this experiment were placed on computer files and sequentially re-combined and named as individual files using a computer program. These files were graphically analyzed using a special modification of the OLD L.IG***:TEKGRAF3 basic program and tektronics computer terminals. Individual water variables and relative fouling amounts were analyzed in an attempt to graphically explain the results obtained.

C. Experiment Two - Exposure of Substrates of Varying Surface Energies to Natural Fouling Organisms

In this phase of the investigation the degree of growth and tenacity of attachment of fouling organisms adhering to substrates with a variety of surface energies were analyzed.

1. Experimental Substrates

The substrates employed were:

(a) Teflon 9000LZ

(b) Hycar Rubber 1022 (Rec. #4) 15' 170C

(c) Polypropylene SPE-SPE-1

(d) Silastic Rubber

(e) CPVC 3401 163-34-80-1

(f) Geon 87324 NAT 021

(g) Dimethyl Polysiloxane

Each of these substances, obtained through the assistance of Thomas Gracik of David W. Taylor Naval Ship Research and Development Center, (NSRDC), was selected based on their established or speculated surface energy values in order to sample substrates of a wide "wettability" range. Listed in Table (2) is each substrate along with its manufacturer, composition, and general description.

TABLE (2) - Experiment Two Substrate Information

Substrate	Manufacturer	General Description
Teflon	Dupont	translucent, colorless, waxy solid
Hycar	B. F. Goodrich	opaque, black, Nitrile rubber
Polypropylene	U. S. Steel	translucent, colorless, hard solid
Silastic	Dow Corning	translucent, colorless, silicon rubber
CPVC	B. F. Goodrich	opaque, ivory, hard solid
Geon	B. F. Goodrich	opaque, off-white, hard solid
Dimethyl Polysiloxane	Dow Corning	transparent, colorless, polymeric liquid

All of the substrates used were of a solid nature with the exception of the Dimethyl Polysiloxane which was a fluid. This necessitated that this substance be irreversibly adsorbed or "cross linked" onto aluminum substrate plates.

Such "cross linking" procedures were done as follows: A large sheet of 0.33 cm thick aluminum was cut into 9.3 cm by 5.3 cm plates. These were washed in a 100% acetone solution to remove any major organic matter (ie. dirt or skin oils). The aluminum pieces, or "coupons" were then placed into the chamber of a glow discharge plasma cleaner which was sealed off and evacuated to 50 millitorr. The coils surrounding the plasma cleaner's chamber strongly ionized the aluminum coupons, facilitating continuous bombardment by atomic particles which stripped away all organic matter left on the plates. This process was continued until a characteristic violet glow was observed which signifies full ionization. The plates were then left in the chamber under full vacuum and ionization for another ten minutes.

Once the aluminum plates had been completely cleaned of all organic materials, they were immediately removed from the plasma cleaner and a small droplet of dimethylpolycyloxane was placed on their surface. Using a lint-free paper (in this instance, lens paper), the plate was rubbed until the coating was a thin even layer, barely visible to the unaided eye. The samples were then placed in an oven preheated to 150 degrees (C) for approximately 14 hours. After baking, the plates were again rubbed to dislodge any

non-bonded coating, leaving only a very thin layer of cross linked dimethyl Polysiloxane.

2. Determination of Surface Energies

As mentioned earlier, the surface energy, or wettability, of a substance is the amount of work required to bring a molecule from a solid's interior to its surface. It is dimensionally equivalent to the surface tension of a liquid. The determination of these values for the substrates investigated was accomplished using a contact goniometer and a computer generated curve-fitting program.

The contact goniometer is a device which allows the measurement of contact angles formed by small droplets placed on a sample substance. The machine consists of a stage which can be moved vertically or horizontally, a focused optical piece complete with a angle measuring scale, and a light source. Illustrated in Figure (7) is a goniometer similar to the Rame'-Hart model used, courtesy of NSRDC.

Samples of each substance to be analyzed were cut to fit the goniometer stage, washed with a mild detergent, and rinsed with distilled water until even water run-off proved them to be free of dirt, oils, and detergent residue. After a thorough air drying, the surface energy for each substrate could be determined.

The substrate to be analyzed was placed on the goniometer stage, and using a flame purified chromium wire a tiny droplet of the first of a set of highly purified liquids was placed upon its surface. The goniometer stage and light

source were adjusted until the droplet was focused and silhouetted, then the right and left contact angles were measured to a half degree using the built-in scale and cursor in the goniometer eye piece. Once the contact angle measurement for the first liquid had been completed, the chromium wire was re-purified and the process was repeated using the remaining liquids. This entire process was then repeated for each substrate. Figure (8) is an example of a contact angle measurement, as seen by the goniometer operator. Table (3) lists the name and surface tension of the set of purified liquids generously supplied by Professor Herman Gucinski of Anne Arundel Community College, Arnold, Maryland.

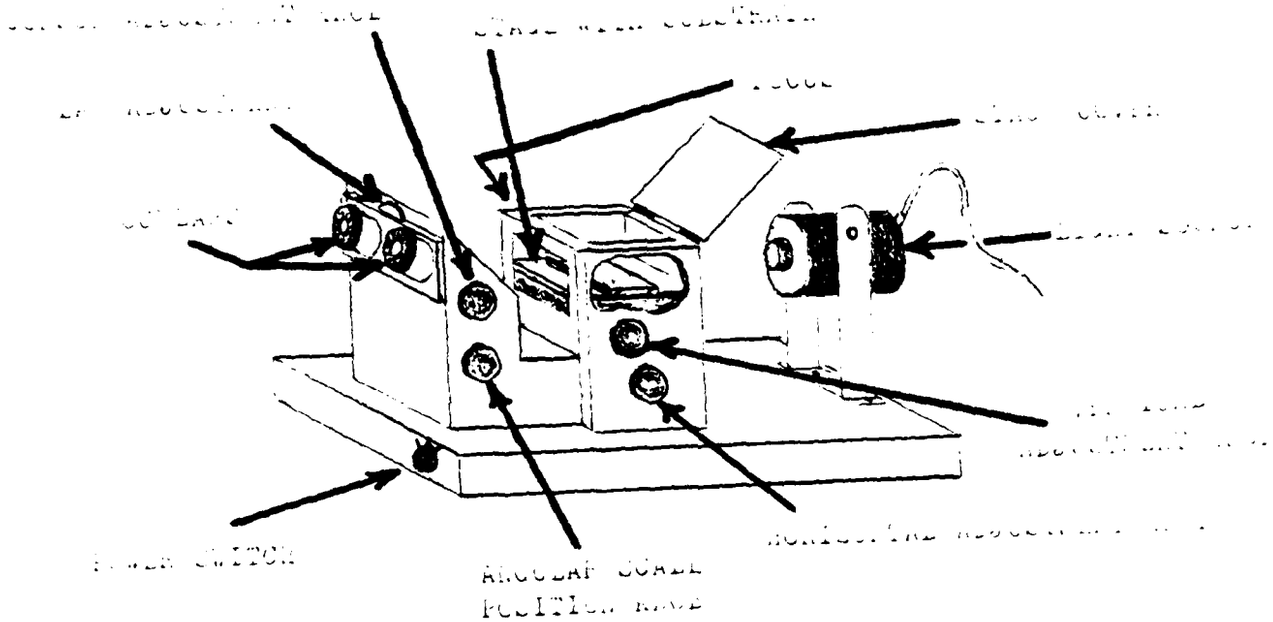


FIGURE (7) -

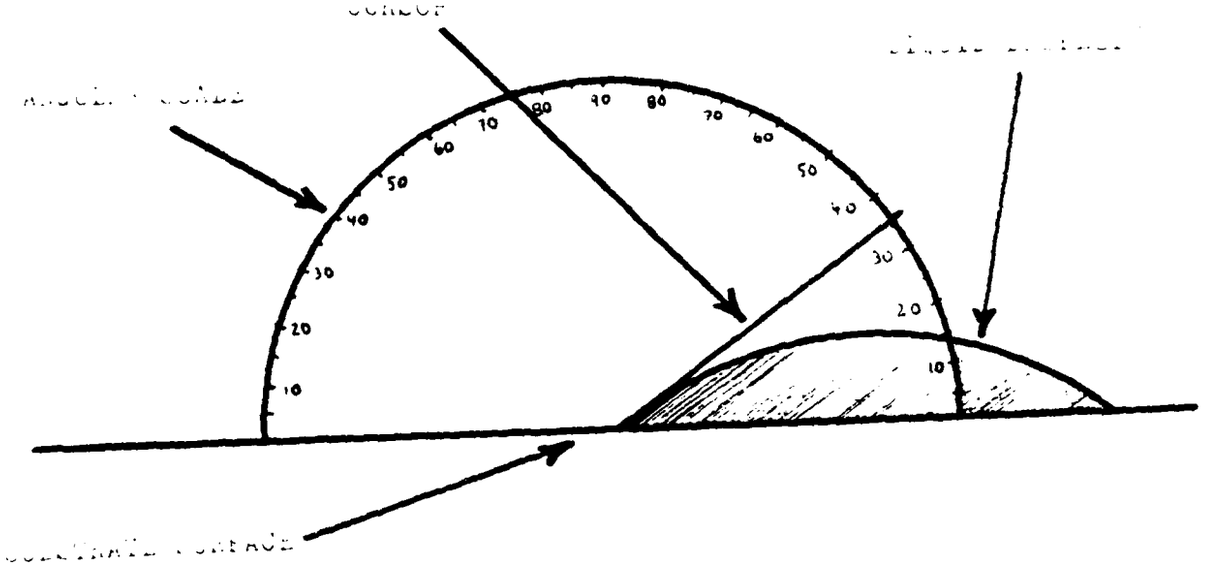


FIGURE (8) -

TABLE (3) - Goniometer Liquids and Surface Tensions

No.	Liquid	Surface Tension (dynes/cm)
1	Water	72.8
2	Glycerol	63.42
3	Formamide	58.2
4	Thiodiglycol	54.0
5	Methylene Iodide	50.8
6	s-Tetrabromeoethane	47.5
7	1-Bromonapthatene	44.6
8	o-diBromobenzene	42.0
9	Tri-o-Cresyl (Po)4	40.9
10	1-Methylnapthtnalene	38.7
11	Dicyclohexyl	33.0
12	n-Hexadecane	27.7
13	n-Tetradecane	26.7
14	n-Tridecane	25.9
15	n-Dodecane	25.4
16	n-Decane	23.9

For each substrate tested, the measured left and right contact angles for each liquid were averaged and placed on a computer file in the form of their cosine values. The respective surface tensions for each liquid were also generated into a computer file, and then the two files were combined so that the cosine of the contact angle corresponded to the appropriate surface tension. These files were graphed using a modification of the CLD L.IG***:TEKGRAF3 computer program, prepared with the assistance of J. Douglas Richardson of the CADIG Rickover hall computing center of the U.S. Naval Academy. Using a built-in least squares curve fitting subroutine, a line was drawn through the cosine/surface tension points and a point where the contact angle cosine was equal to one (or a contact angle of zero) was interpolated. The corresponding surface tension value was then designated as that substance's surface energy or wettability.*

3. Preparation and Exposure of Substrates

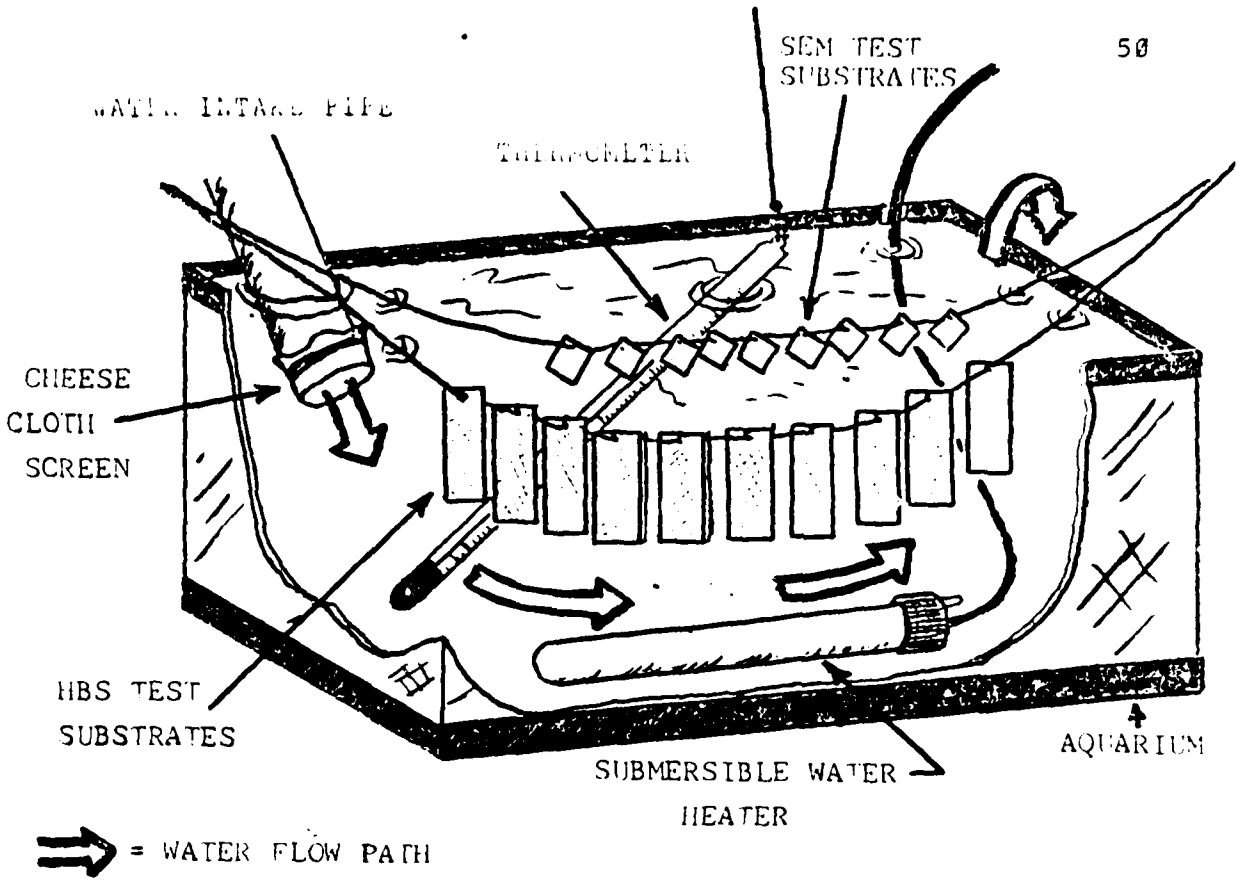
The substrates to be analyzed were cut into sixteen pieces; eight measuring 8.6 cm by 2 cm and eight measuring 1 cm by 1 cm. The larger plates were drilled with a 0.7 cm hole centered approximately 1.3 cm from the top of one narrow end, into which a wire support hook was inserted. A length of 0.7 cm nylon line was run through the eye of each clip and a knot was tied to keep the samples in position.

* As described in Baier, 1977.

The smaller samples were drilled with a 0.17 cm hole in one corner, into which a length of common thread was inserted. Knots were tied in the thread at each sample to keep them separate. The ends of the threads were to a wire frame which ensured that the thread remained relatively tight and the samples separate.

Eight of each size samples were suspended in one of two flow-through flushing aquariums located in the Oceanographic Pier Laboratory of the Naval Academy. The aquaria were placed in a large holding tank which had a water inflow pipe for each aquarium and one central drain. The two tanks differed only in that number one had a piece of fine mesh (#20) plankton net stretched across its inflow pipe opening, preventing larger fouling organisms such as animal larvae and crustaceans from entering the aquarium system, whereas tank number two had no such screening.

Severn River water was pumped through the intake piping directly into each tank where it was gradually warmed by two 200 watt submersible heaters. By regular adjustments of the heater controls and the water flow rates, a constant temperature of between 16 and 19 degrees (C) was maintained throughout the exposure period of 90 days. The warmed water increased the metabolic rates of the existing fouling organisms, thus making it possible to expose substrates to rapid rates of biological fouling. Illustrated in Figure (9) is aquarium number one showing substrates and water flow path.



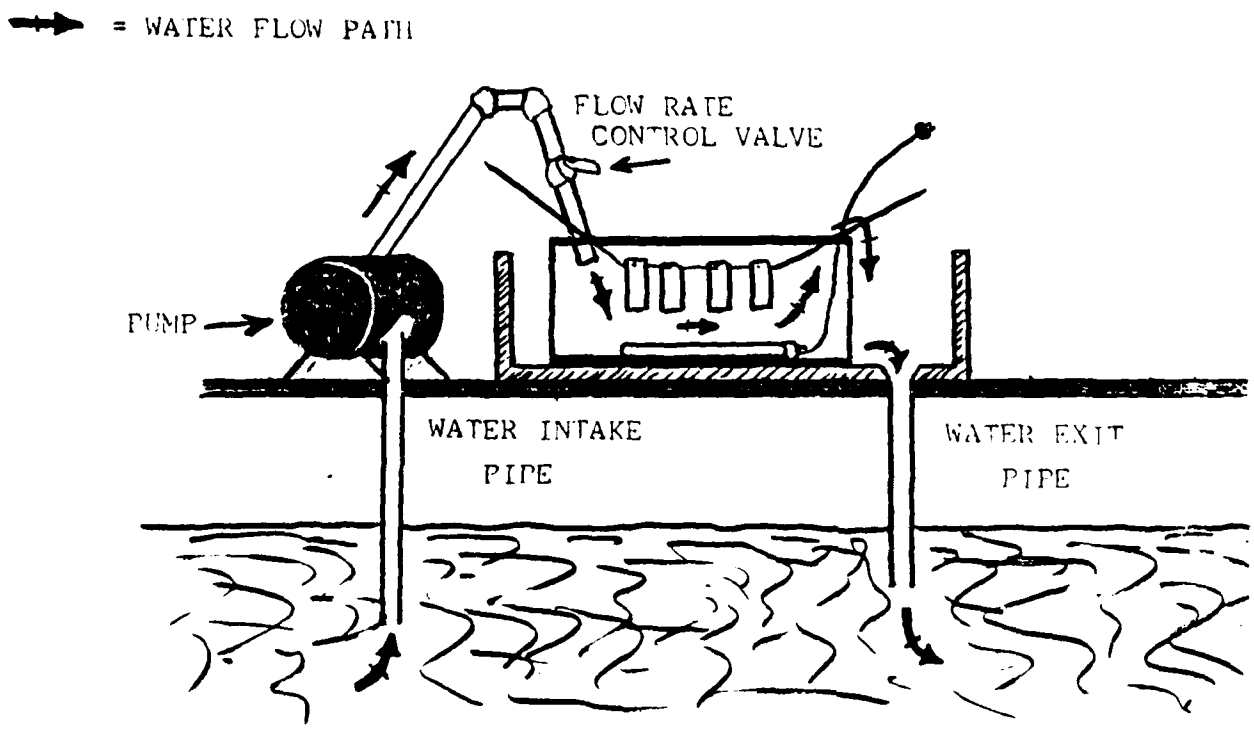
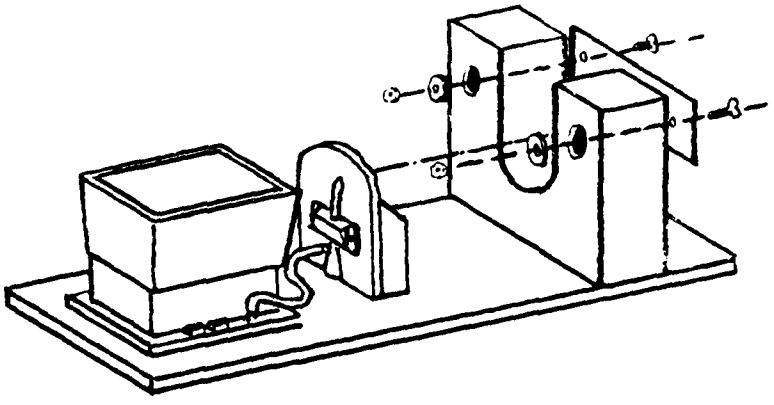
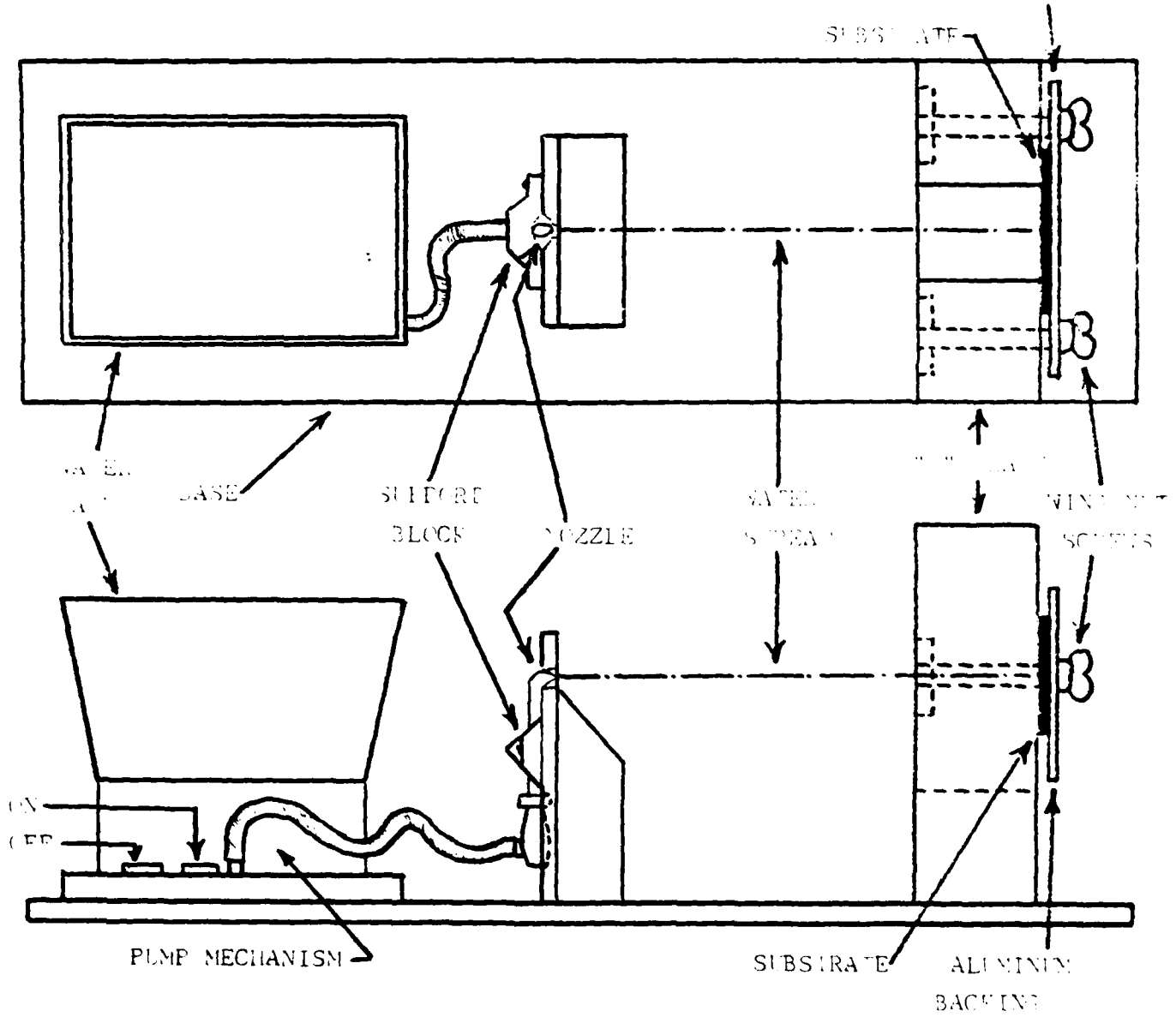


FIGURE (9) - EXPOSURE AQUARIUM SYSTEM

4. Analysis of Substrates

Since all of the substrates investigated were opaque in nature, normal microscope analysis, such as that used in experiment one, was not possible. Three sets of the larger samples were examined under a dissection microscope at 500 times magnification and cross illumination. Clusters of fouling organisms were counted by three individual tests which randomly sampled 10, 15, and 30, 3.2 square millimeter fields of view. Once organism cluster counts had been taken, the substrates were exposed to shear from the spray of the Hydrous Bacterial Sprayer (HBS), which was designed and built especially for this experiment and is shown in Figure (10). Substrate plates were securely clamped into the holding "U" and submitted to between 10 and 60 seconds of a pulsating tap water stream from the sprayer's nozzle. Two sets of samples received direct water from the sprayer, while the third set had the water stream dampened by a fine mesh (#20) plankton net set between the sprayer and the holding "U". Once HBS exposure had been completed, the substrates were re-examined under the dissection microscope and counts of remaining organic materials made. The results of these and the earlier counts were then placed onto computer files, averaged using a BASIC computer program, [illegible]d with their respective substrate surface energies, and [illegible]aphed using a Xynetics plotting system.




SUBSTRATE
BASE
SUPPORT
BLOCK
NOZZLE
ON
OFF
PUMP MECHANISM
SUBSTRATE
ALUMINUM
BACKING

The four smaller sets of substrates were prepared for HBS testing by securely fastening a piece of clear acetate over approximately one-half of their frontal surface area. The substrates were then placed into small, wooden supports, secured into the holding "U" of the HBS, and exposed to 90 seconds of a direct water stream hitting their uncovered half. This procedure is illustrated in Figure (11). Once HBS exposure had been completed, the substrates were marked with a razor line to clearly distinguish between their exposed and unexposed sides, and allowed to dry for 48 hours.

Once completely dry, the substrates were ready for processing in preparation for Scanning Electron Microscope (SEM) analysis. Since the image produced on this device originates from electrons being reflected off of the subject or "target" and hitting the collector or "screen", special processing procedures become necessary. As Kelly (1981), citing Hayat (1978) wrote:

> Nonconductive specimens ... cannot rapidly channel the excess primary electrons away from the scanned area and a local charge may build up on the specimen's surface. This increases abnormally the secondary electrons emitted to the collector and creates a localized glow which destroys the imaging of the microscope.

Kelly went on to state that to avoid this situation, an extremely thin coating of a conductive material, such as carbon or gold, had to be applied to the surface of the samples.

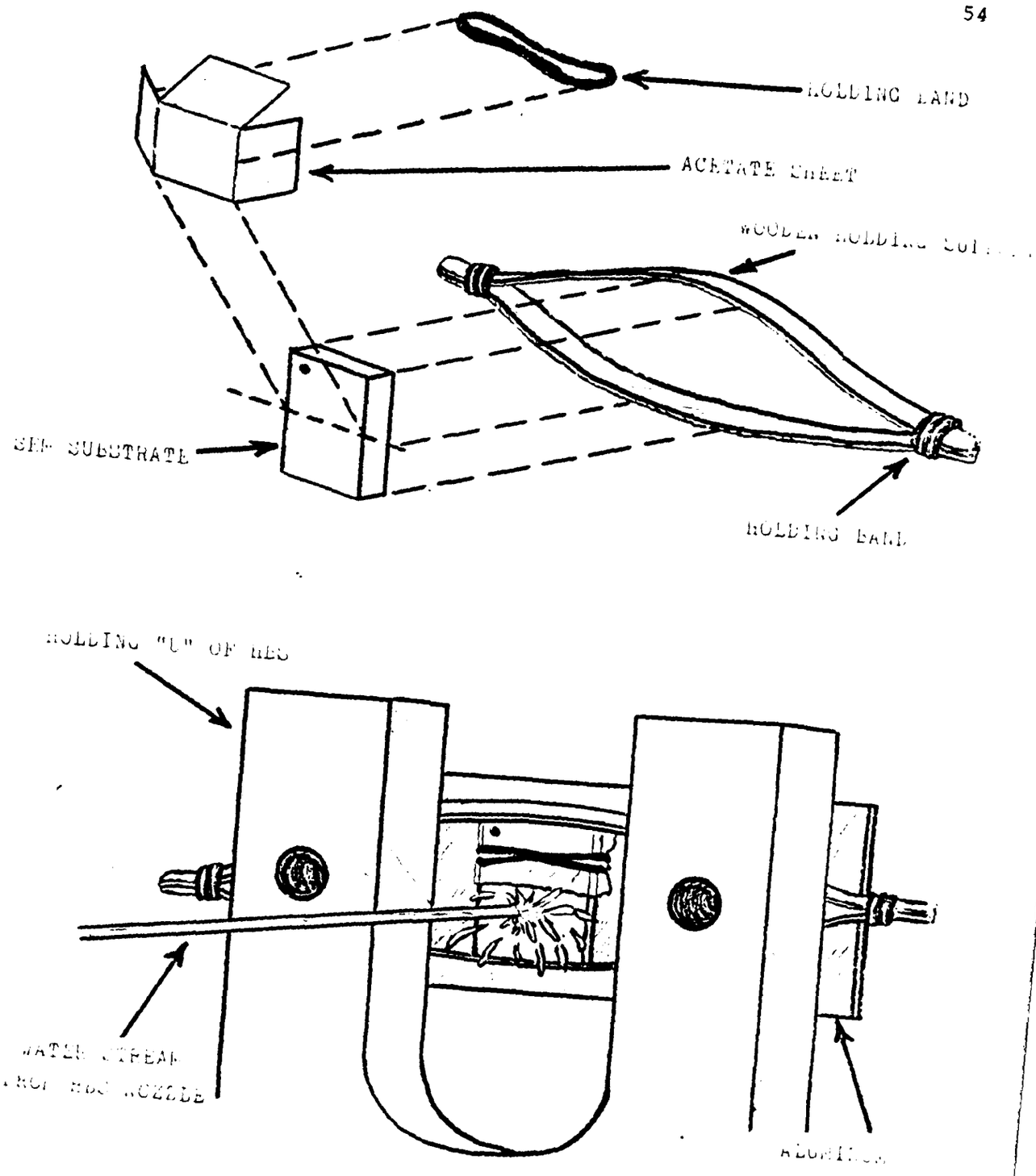

HOLDING BAND
ACETATE SHEET
SEM SUBSTRATE
HOLDING BAND
HOLDING "U" OF HBS
WATER STREAM
FROM HBS NOZZLE

FIGURE (11) - [illegible]

In preparation for the conductive coating process, each of the substrates was fastened onto a small aluminum pedestal which allowed ease of handling and storage during analysis. One-half of the head of each pedestal was coated with a fixative glue, and the other half with a conductive carbon liquid. The substrate sample was then firmly pressed onto the head surface, and a thin streak of liquid carbon was run from the "carboned" side of the substrate bottom to the upper edge of the substrate surface in order to ensure complete coating. After the liquid carbon had dried for two hours, the mounted substrates were placed into the holding chamber of a conductive coating machine made by the Technics Corp. of Alexandria, Virginia. This chamber was initially purged with Argon gas, and then evacuated to 50 millitorr. Substrates were coated by electromagnetically vaporizing part of a disc of gold pallatium placed in the top of the chamber. This vaporization caused the ionized gold pallatium particles to bombard the substrate samples, covering them with a thin conductive coating. The coating process was continued for three minutes, giving the samples a layer between 200 to 300 angstroms thick. Once all substrates had been coated, they were individually placed into the chamber of the SEM and comparative analysis of the degree of fouling remaining on their "before" and "after" HBS testing sides was conducted. During this analysis, random, representative Polariod photographs of each of these sides, along with close-up photographs of representative or especially

interesting organisms seen were made. Each set of "before" and "after" photographs were compared and ranked on the basis of degree of fouling shown. The photograph demonstrating the highest degree of fouling was designated a number of twenty, while the one with the lowest was given a number one. All remaining photographs in each "before" or "after" set were designated a relative fouling number between one and twenty, based on their individual degree of fouling shown. These values were then graphically compared to the substrate surface energies, as determined through the contact goniometer tests, in order to discern if any relation between these values (ie. degree of fouling vs. surface energy, degree of organism hold vs. surface energy) could be determined.

D. Experiment Three - Analysis of Water Components Necessary to Promote Bacterial Transfer

1. Collection of Bacterial Samples

In order to obtain samples of pioneer periphytic bacteria, a set of twelve precleaned 7.5 cm by 2.5 cm microscope slides were placed and sealed into a standard slide box which had been modified as previously described in section 1 of Experiment 1 Materials and Methods. The box was immersed in flushing tank number one, (Figure (8)), set up in the oceanographic pier lab at the Naval Academy. The box was removed, after 24 hours of submergence, from the tank, each of its slides was removed and individually rinsed with sterile Severn River water to dislodge any loose debris.* A sample of Severn River water was also collected at this time.

2. Preparation of Artificial Sea water Solution

The artificial sea water formula of Lyman and Flemming (1934) was used in this experiment. A listing of this formula, as presented in Sverdrup, Johnson, and Flemming (1942), is shown in Table (4). Three individual batches of sea water solutions were made from this formula for this investigation. For each, twice the amount of each salt components listed in Table (4) was weighed out to the nearest 1/100 gram and poured into a 4000 ml flask containing 2000 ml of distilled water which had been sterilized by 20

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* As described in Clopp, 1981.

minutes exposure at 120 degrees (C) in an Autoclave unit, or, depending upon the batch, by use of a millipore filter system. The "double batch" solution was then thoroughly mixed for one hour using a magnetic stirrer, at which time 500 ml were withdrawn and mixed with 500 ml of sterile distilled water, creating a one-half cut (salinity of approximately 16 ppt) solution.

TABLE (4)

FORMULA FOR ARTIFICIAL SEA WATER

Salt	g/kg
NaCl	23.476
MgCl2	4.981
Na2SO4	3.971
CaCl2	1.102
KCl	0.664
NaHCO3	0.192
KBr	0.096
H3BO3	0.026
SrCl2	0.024
NaF	0.003
Total	34.481
Water to	1,000.000

3. Analysis of Solutions for Bacterial Transfer

A total of six bacterial transfer "runs" were performed in which approximately 75 ml of each of a set of sample solutions was poured into a sterile Coplin jar containing two standard precleaned microscope slides, positioned at opposite ends of the jar's slide retaining grooves. Each set of solutions contained several samples of artificial sea water which had been organically enriched, and a sample of straight artificial sea water (stock or half-cut strength), or a sample of distilled water as a control. Several samples of artificial sea water which had been organically enriched. A bacterially "fouled" slide, (ie. a slide which had been exposed to fouling bacteria for sufficient time to cause bacterial attachment to occur) was then placed in the center of each Coplin jar's slide holder, in between the two precleaned slides, and the samples were left undisturbed for 48 hours. Initial "fouled" slides used were those taken directly from the slide box which had been suspended in Severn River water, but later runs of the experiment used slides which had been bacterially fouled during the previous runs. This ensured that the bacteria used in these runs were all periphytic fouling types which would colonize slides, and not simply bacteria present in the Severn River water droplets on the initially exposed slides. A complete list of each set of solutions used in the six runs is listed in Tables (5) through (10). Once the 48 hour exposure period had passed, one of the precleaned slides from each

jar was removed and immersed for five minutes in a crystal violet stain solution. Each slide was rinsed twice with distilled water, blotted dry using an ordinary paper towel, and viewed at high power (600 X magnification), to determine if any bacteria had transferred from the fouled slide to the clean slide, through the solution. Samples were scanned and given a numeric designation as follows:

0 - no bacteria present
1 - few scattered bacteria present
2 - many scattered bacteria present
3 - few bacterial colonies present
4 - many bacterial colonies present

4. Sterilization of Glassware

To prevent bacterial contamination of artificial sea water solutions, all glassware was thoroughly cleaned and sterilized prior to use. Flasks, beakers, graduates, and the millipore filter system were steam sterilized in the autoclave unit as described earlier. The coplin jars, which were subject to cracking during autoclaving, were sterilized by soaking for ten minutes in a 95% Ethanol solution, rinsing three times with sterile distilled water, inverting until dry, and capping to prevent intrusion of any microorganisms.

TABLE (5) - Analysis of Solutions Containing Proteins
(Trial #1)

Soln. #	Contents (*)
1	Severn River water
2	Severn River water (sterile)
3	artificial sea water (sterile)
4	artificial sea water (1/2 salinity) (sterile)
5	artificial sea water (1/2 salinity) + .005 % Peptone
6	artificial sea water (1/2 salinity) + .05 % Peptone
7	artificial sea water (1/2 salinity) + .005 % Yeast
8	artificial sea water (1/2 salinity) + .05 % Yeast

Source of "fouled" slides = Severn River

TABLE (6) - Analysis of Solutions Containing Proteins
(Trial #2)

Soln. #	Contents (*)
1	Severn River water (sterile)
2	distilled water (sterile)
3	artificial sea water (1/2 salinity) (sterile)
4	artificial sea water (1/2 salinity) + .005 % Peptone
5	artificial sea water (1/2 salinity) + .05 % Peptone
6	artificial sea water (1/2 salinity) + .005 % Yeast
7	artificial sea water (1/2 salinity) + .05 % Yeast

Source of "fouled" slides = Equivalent Soln. from Trial #1

* percentages given by weight

TABLE (7) - Analysis of Solutions Containing Sugars
(Trial #1)

Soln. #	Contents (*)
1	distilled water (sterile)
2	artificial sea water (1/2 salinity) (sterile)
3	artificial sea water (1/2 salinity) + .005 % Sucrose
4	artificial sea water (1/2 salinity) + .005 % Galactose
5	artificial sea water (1/2 salinity) + .005 % Glucose
6	artificial sea water (1/2 salinity) + .005 % Lactose

Source of "fouled" slides = .05 % Peptone

TABLE (8) - Analysis of Solutions Containing Sugars
(Trial #2)

Soln. #	Contents (*)
1	distilled water (sterile)
2	artificial sea water (1/2 salinity) (sterile)
3	artificial sea water (1/2 salinity) + .005 % Sucrose
4	artificial sea water (1/2 salinity) + .005 % Galactose
5	artificial sea water (1/2 salinity) + .005 % Glucose
6	artificial sea water (1/2 salinity) + .005 % Lactose

Source of "fouled" slides = Equivalent Soln. from Trial #1

* percentages given by weight

TABLE (9) - Analysis of Solutions Containing Vitamins
(Trial #1)

Soln. #	Contents (*)
1	distilled water (sterile)
2	artificial sea water (1/2 salinity) (sterile)
3	artificial sea water (1/2 salinity) + .005 % Vitamin B
4	artificial sea water (1/2 salinity) + .005 % Lysozyme
5	artificial sea water (1/2 salinity) + .005 % Niacin
6	artificial sea water (1/2 salinity) + .005 % Vitamin B6

Source of "fouled" slides = .05 % Peptone Solution

TABLE (10) - Analysis of Solutions Containing Vitamins
(Trial #2)

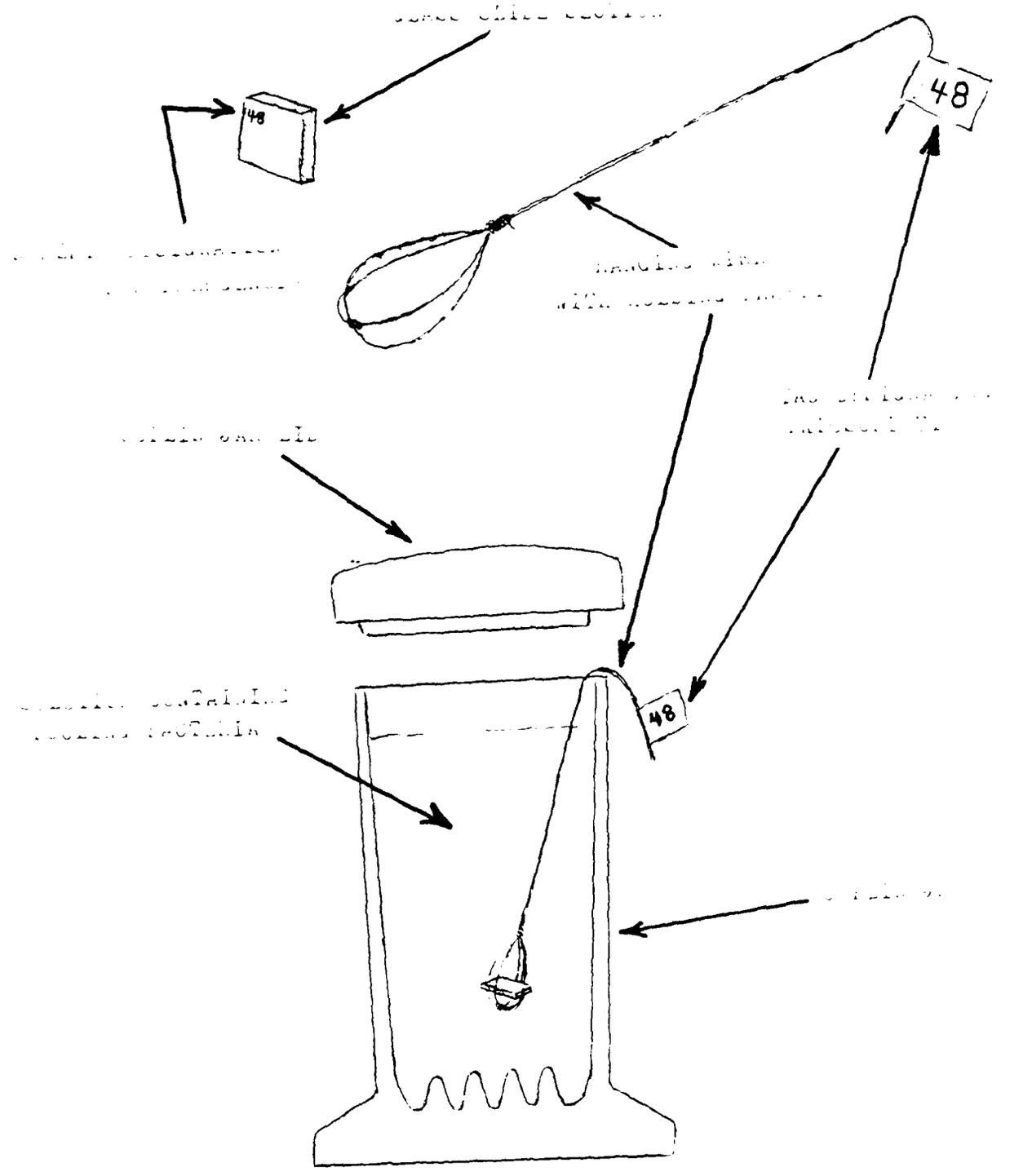
Soln. #	Contents (*)
1	distilled water (sterile)
2	artificial sea water (1/2 salinity) (sterile)
3	artificial sea water (1/2 salinity) + .005 % Vitamin B
4	artificial sea water (1/2 salinity) + .005 % Lysozyme
5	artificial sea water (1/2 salinity) + .005 % Niacin
6	artificial sea water (1/2 salinity) + .005 % Vitamin B6

Source of "fouled" slides = Equivalent soln. from Trial #1

* percentages given by weight

5. SEM Analysis of Initial Bacterial Fouling

Precleaned glass microscope slides were cut into 0.7 cm by 0.7 cm squares. Using a diamond scratching tool, the first two sections were marked 0 (zero) and 12, while subsequent sections were marked from 24 to 144 in increments of 24. Each numbered square was fitted into a wire "exposure basket", and submerged into a bath of boiling distilled water for ten minutes for bacterial sterilization. Each slide was then suspended by a length of wire attached to its "exposure basket" in a solution of artificial sea water enriched with fouling bacteria from the second run of the experiment. This process is illustrated in Figure (12). After twelve hours, the slide marked "12" was removed from the jar and rinsed with distilled water to remove any residual salt solution, and allowed to air dry under an inverted beaker. Each remaining slide section was sequentially removed, based on its numeric marking, rinsed, and dried in the same manner. Once all slide sections (less the one marked "0") had been exposed, rinsed, and thoroughly dried, they were prepared for SEM analysis by being glued and carbon fixed on to an aluminum pedestal, and coated with gold pallatium, as described in section 9 of experiment two materials and methods. Using the SEM, each fouled section was observed and photographs taken of any pioneer periphytic bacteria present.


48
48
48

IV. Results

A. Experiment One

1. General

Two of the seven sampling stations established on the Severn River (Figure (3)) were functionally destroyed during the 14 day sampling period. High waves caused by a thunderstorm on 19 October, 1982, broke open slide boxes 1 and 2 (Station (1)) located at a small pier on Bay Ridge Beach. Water samples were taken at this station on the first, third, and seventh sampling days, but since all microscope slides were lost when the boxes broke open, no fouling samples were obtained. Slide boxes eight and nine (Station (5)), suspended off a pier at the Severn Prep School Beach, disappeared between the tenth and fourteenth sampling days, preventing a day 14 fouling sample from being collected. water variables were measured, and, for graphical analysis purposes, a relative fouling number of 5.5, based on past fouling trends for that station, was designated for that sampling day. All other stations remained functional throughout the 14 day sampling period, however, station two, located at the "Spider Buoy", was reduced to only 1 sampling box by a thunderstorm on 21 October, which destroyed slide box number three. Slide box number 4, also located at station two, was reinforced with additional holding bands and remained functional for the duration of the sampling period.

2. Graphical Analysis of Water Quality

Depicted in Appendix A, Figures (13) through (40), are water variables as graphed against time for each of the seven stations. The graphs are separated on the basis of water variables as follows:

Figures (13) through (19) - Salinity vs. Time
Figures (20) through (26) - Conductivity vs. Time
Figures (27) through (33) - Oxygen vs. Time
Figures (34) through (40) - Temperature vs. Time

All graphs show five data points for the five sampling days, with the exception of the graphs for station 1 which depict only water variable data from the first, third, and seventh sampling days, as stated.

3. Graphical Analysis of Relative Fouling

Illustrated in Appendix B, Figures (41) through (46), are graphs of fouling vs. time at stations 2 through 7, respectively. The relative fouling values for each were assigned in the manner discussed in section 5, "Analysis Procedures" of the Materials and methods section of this report. A composite graph of fouling vs. time for all stations is shown in Figure (47).

4. Observations Based on Graphed Data

Figures (13) through (47) were analyzed and counts were made of the frequency of an individual station experiencing the highest, second highest, lowest, and second lowest data values for the water variables shown. In a situation where two water variable values were of the same value, each was

counted as one half. Shown in Table (11) are the results of these counts.

TABLE (11) - Summary of Experiment One Observations

Station #	2	3	4	5	6	7
Number of times:						
Relative Fouling:						
Highest	0	2	1	0	0	2
Second Highest	0	0	2	0	0	3
Lowest	5	0	0	0	0	0
Second Lowest	0	2	0	1.5	1.5	0
Salinity:						
Highest	3	0	2	0	0	0
Second Highest	0	4	0	1	0	0
Lowest	0	0	0	0	1	4
Second Lowest	0	0	0	0	4	1
Oxygen:						
Highest	0	0	0	2	3	0
Second Highest	0	0	1	1	1	2
Lowest	1.5	1.5	1	1	0	0
Second Lowest	2	1	1	1	0	0
Conductivity:						
Highest	4	0	1	0	0	0
Second Highest	0	4	0	1	0	0
Lowest	0	0	0	0	0	5
Second Lowest	0	0	0	0	5	0
Temperature:						
Highest	2	0	1	1	1	0
Second Highest	0	1	1	2	0	1
Lowest	2	2	0	0	0	1
Second Lowest	1	2	0	0	2	0

Based on the data shown in Table (11), the following observations listed in Tables (12) through (16) were made:

TABLE (12) - Topic: Salinity

Occurrence	Station Number(s)	Frequency (%)
highest	2	60
second highest	3	80
highest	2,3, or 4	100
highest, second highest	2,3,4, or 5	100
lowest	7	80
second lowest	6	80
second lowest	7	50

TABLE (13) - Topic: Conductivity

Occurrence	Station Number(s)	Frequency (%)
highest	2	80
second highest	3	80
highest, second highest	2 or 3	80
highest, second highest	2,3, or 4	90
lowest	7	100
second lowest	6	100

TABLE (14) - Topic: Oxygen Content

Occurrence	Station Number(s)	Frequency (%)
highest	6	60
highest	5	40
highest, second highest	5 or 6	70
lowest, second lowest	2	35
lowest, second lowest	2 or 3	60

TABLE (15) - Topic: Temperature

Occurrence	Station Number(s)	Frequency (%)
highest, second highest	4	30
highest, second highest	4 or 5	50
lowest	2	40
second lowest	3	40
lowest, second lowest	3	40
lowest, second lowest	2	30
lowest	2 or 3	80
second lowest	2 or 3	60
lowest or second lowest	2 or 3	70

TABLE (16) - Topic: Relative Fouling

Occurrence	Station Number(s)	Frequency (%)
highest, second highest	7	50
highest, second highest	4 or 7	80
lowest	2	100
lowest, second lowest	2 or 3	70

Once observations had been made, new computer files were created by combining water variables and relative degrees of fouling for particular dates and stations. Shown in Figures (48) through (67) of Appendix C are graphs which depict this information as follows:

Figures (48) through (52) - Salinity vs. Fouling
Figures (53) through (57) - Conductivity vs. Fouling
Figures (58) through (62) - Oxygen vs. Fouling
Figures (63) through (67) - Temperature vs. Fouling

Based on these graphs, the following general observations were made:

Graphs of Salinity vs. Relative Fouling :

Low (4 to 6 ppt) to intermediate (12 ppt) salinities experienced the highest degrees of relative fouling, while higher salinities (+13 ppt), generally experienced the least. Exceptions to this occurred on sampling days 7 and 10.

Graphs of Conductivity vs. Relative Fouling :

Conductivities in the 17 ohm range were, with one exception, the values which corresponded to the largest degree of relative fouling for each graph. However, extremely low fouling values appear within this range also, especially on graphs of days 10 and 14. On day 7, the peak fouling value occurred at a conductivity value of 13.58 ohms, with a minor peak occurring with the 17 ohm range.

Graphs of Oxygen Content vs. Fouling :

The highest relative fouling values on each sampling day occurred at a varying degree of oxygen content values. Generally, low oxygen content stations experienced relatively low degrees of fouling.

Graphs of Temperature vs. Relative Fouling :

Several graphs exhibited a number of relatively high fouling values or "peaks" over a range of temperatures. The highest relative fouling value on each day generally occurred at temperatures in the 15 to 18 degrees (C) range. The exception to this occurred on day 10 where the peak fouling value occurred at 13.6 degrees (C).

5. Fouling Organisms Observed

Organisms observed and photographed using the Leitz Wetzler microscopic camera system were identified through descriptions, illustrations, and photographs shown in Jahn (1949), Griffith (1961), Green (1971), and Bick (1972). After the first day of submerged exposure, pioneer periphytic bacteria were observed to have colonized the glass substrate surfaces at all sampling sites. At stations 2, 3, and 4, these bacteria had established large chains and numerous initial colonies. Bacteria were also present and numerous at other stations, but were more scattered and lacked colonial organization. Several types of protozoans and attached phytoplankton, or "Periphyton", were also found on the slide samples located at stations 4 and 5. The protozoans present were primarily long, narrow, flagellated, often twisted, members of the family "Euglenidae", specifically, Euglena Gracilus, Euglena Ehrenbergii, or Euglena Spirogyra, and stalked, round budded Vorticella Monilata of the tribe Aloricata. Also present were slender, tube-like Ascoglena of the genus Ascoglena, and the round gelatin-like Palmella stages of Colaciium Vesiculatum, of the family Colaciidae. The periphyton observed at stations 4 and 5 after 1 day of exposure were primarily barrel-shaped members of the family "Tabellariaceae", such as Rhabdonema, Stiatella, and possibly members of the family "Diatomaceae", sucn as Plagiogramma.

After 3 days of submergence, slide samples at several of the stations had advanced significantly in the fouling succession process. Station 2 developed large, circular bacterial colonies, held firmly to the substrate surface by strands of what appeared as the mucopolysaccharide glue discussed earlier. At station 3 many bacterial chains, and also a large number of stalked, bell-shaped protozoans identified as possibly Vorticella or Zoothamnium, both of the tribe "Aloricata", were observed. These organisms were surrounded by heavy masses of organic debris, entangled in the cilia on their bodies.

Stations 4 and 5 exhibited the same types of protozoans and periphyton that had been observed after the initial submergence day, but two significant changes were also observed. At station 4, the gelatinous Palmella stages of Colaciium Vesiculatum had advanced to both the free-living flagellated and the sessile, stalked, binary budded stages of that organism. These, along with more Palmella stages had become the dominant organism over the entire surface at that site. Additionally, needle-like periphyton of the family "Fragilanceae", specifically, Flagillaria, Synedra, Asterionella, and Thalassiothrix, and chains of barrel-shaped cells belonging to Ulothrix, of the family "Ulotrichaceae", became very common at both stations 4 and 5.

Stations 6 and 7 exhibited many masses of organic matter scattered among, and attached to the same types of protozoans and periphyton found at stations 4 and 5, however, both of these stations seemed to be dominated by the stalked, bell-shaped protozoans Vorticella and Vorticella Campanella. These two stations also contained heavy amounts of organic matter which had collected on, or around, the fouling organisms present.

By the seventh day of exposure, all stations demonstrated large, well-established bacterial colonies in the form of spherical or matted clusters which covered the majority of the exposed surface area. At all stations, colonies of stalked, budding protozoans belonging either to the tribe "Aloricata", specifically, Vorticella Monilata, Carchesium Polypinum, or Zoothamnium Adamsi, or to the family "Colaciidae", specifically, Colaciium Vesiculatum, became the dominant fouling organism. This dominance was especially obvious at station two, which had shown bacteria in small colonies up through the third day of exposure. By the seventh day, however, the sampling slides at this station had become almost completely covered by the stalked protozoans mentioned earlier.

The sample slides at stations 4, 5, 6, and 7 also contained several species of periphyton, such as diatoms belonging to the order "Centrales", such as Cosconodiscus, Planktonella, Actinopyychus, and Actinocylus, colonial Periphyton such as Asteionella and Thalassiothrix of the family

"Fragilariance", and Actinastrum, of the family "Scenidesmaceae", and individual periphyton such as Pennularia and Tropidoneis, of the family "Naviculaceae", and Nitzschia, of the family "Nitzschiaceae".

By the tenth and fourteenth days of submergence, the types of individual fouling organisms present at each of the stations had changed, visibly, very little. However, the number of organisms and amount of organic debris present had increased substantially. The stalked, budding protozoans remained the dominant of the "larger" fouling organisms, but by these latter sampling days (10) and 14), they had become so engulfed and entangled in themselves and other fouling materials present, that distinguishing individual organisms, or even individual colonies of organisms, became nearly impossible.

B. Experiment Two

1. Substrate Surface Energy Determination

A series of two goniometer tests were performed on each of the seven substrates listed in Table (2). The first, to acquire familiarity with the materials and procedures used, and the second, to collect actual data for subsequent analysis. Experience in operation of the contact goniometer was necessary to ensure accurate results because simple errors such as improper focusing, or not allowing droplets to fully settle could change a contact angle measurement by as much as 5 degrees.

During the initial goniometer testing, it was discovered that several of the substrates, especially hycar rubber, CPVC, and Geon, were absorbent to certain of the test liquids listed in Table (3). In these instances, liquid droplets placed on the substrate surface would gradually be absorbed, decreasing both in size and contact angle. Under these circumstances, accurate contact angle measurements were not possible. To correct this problem, measurements taken from liquids observed to absorb into a certain substrate were eliminated from subsequent analysis to determine that samples surface energy. This created a varying number of data values (usually between 8 and 12) used in subsequent graphical analysis. It was also discovered, during the initial goniometer work, that test liquid (1), distilled water, had been contaminated. This made measurements using this liquid erroneous and inaccurate. Once determined

to be contaminated, distilled water was not used in later testing, and earlier measurements using this liquid were disregarded.

Once sufficient familiarity of the proper contact angle measurement procedures, such as cleaning of substrates, liquid/substrate interactions, and actual practice time on the goniometer, had been attained, the second test of the substrates was conducted to collect contact angle measurements for surface energy determination. As mentioned previously, these angular values were combined and graphed using a modified version of TEKGRAF3 BASIC computer program to produced graphs of surface tensions vs. contact angle measurements which followed the basic format of Bier, (1977). Appendix D, Figures (68) through (74) presents these graphs. From the equation of the straight line which was "fitted" through the points, the substrate surface energy was determined, as described in section 7 of Experiment two Materials and Methods. Listed in Table (17) are the surface energy values determined by this method for both the initial and secondary goniometer tests. Also listed in Table (17) are the "established" surface energy values, as determined by previous investigators. The pre-determined surface energy values listed for Silastic and Hycar rubber are given as a range of possible values because no specific surface energies for these substances have been determined and published to date. Also, because these substances are a complex mixture of various polymeric materials, actual ingredients of

individual batches may differ, causing a range of surface energy values (Griffith, 1983).

TABLE (17) - Substrate Surface Energies

Substrate Name	Determined Value (dynes/cm) Test #1	Test #2	Established Value (dynes/cm)
Teflon	21.7	19.9	19.3 *
Dimethyl P.	24.6	23.1	22.5 **
Silastic	25.3	27.5	27-28 ***
Polypropylene	30.4	28.9	30.7 *
Hycar	36.3	36.7	35-40 ***
Geon	38.1	40.2	43.0 *
CPVC	36.8	41.1	43.0 *

* Becka, 1981
** Dexter, 1977
*** Griffith, 1983

2. Analysis of Fouling Using HBS and Dissection Microscope

After the 90 day exposure period, the large (8.6 cm by 2 cm) substrate samples demonstrated a heavy degree of fouling, creating a well established "secondary film" layer. Three series counts of colonies present on each of the seven substrates analyzed were performed. In the first series, 10 fields of view, 3.2 centimeters square, were counted. In the second series, 15 fields of view, and in the third series 30 fields of view were counted. After the primary or "before" counts had been made, the first (10 count) set of samples was exposed to 20 seconds of unobstructed water stream from the HBS nozzle, located 15 cm from the substrate's surface. Upon subsequent analysis under the dissection microscope, it was discovered that the HBS had removed all visible fouling organisms from the substrates' surfaces, thus preventing the counting of remaining fouling organisms for this set of samples. The next (15 count) set of substrates were exposed to 10 seconds of unobstructed water stream from the HBS nozzle, located 40 cm from the substrate surface. Subsequent analysis of these samples showed that, even at the decreased exposure time and increased distance, all of the visible fouling organisms had been removed from the substrates' surfaces, preventing "after" exposure counts on these samples as well. The third set of samples (30 count) were exposed to 10 seconds of water stream from the HBS nozzle, located 40 cm from the substrate's surface, however, for these tests, a piece of

fine mesh (#20) plankton netting was placed between the nozzle and each substrate's surface. This netting acted as an obstruction to the water streams path, diffusing its concentration and decreasing its dislodging force. Observations by dissection scope of these samples showed some fouling organisms to be remaining after exposure. These organisms were used for "after" exposure organism counts.

Once all counts were completed and substrates exposed, the collected data were averaged, combined with their respective surface energies, and graphed as described earlier. The resultant graphs of this data are presented as Figure (75) through (78) of Appendix E.

3. Analysis of Relative Fouling Using HBS and SEM

The set of 8 smaller (1 cm by 1 cm) samples were partially exposed to 90 seconds of a direct water stream from the HBS nozzle, located 20 cm from the substrate's surface. Longer exposure time and less distance were utilized, since in this analysis using the SEM, it was not necessary to have large (visible with a light microscope) colonies present, but only tiny microfouling organisms or their remnants. The "before" and "after" HBS exposure photographs, shown in plates (1) through (14) of Appendix F, were ranked on a scale of 1 to 20, combined with their respective substrate surface energies, and graphed in the manner described previously. The graphs of this data are shown in Figures (79) and (80) of appendix G.

4. Observations Based on Graphed Data

The set of "before" HBS exposure graphs, both based on dissection microscope counts, and on SEM photograph analysis, were compared to the results described in Baier (1977, and Dexter (1978), as discussed in section 3 of the introduction, and to the graph listed in Figure (3),in which Baier (1977) related substrate surface energy to fouling experienced. The following observations were made:

(1) Two of the substrates analyzed, Dimethyl P. and Silastic, had surface energies within the 20 to 30 dynes/cm, "minimal fouling" range described by Baier.

(2) Only one substrate, Dimethyl P. had a surface energy within the 22 to 24 dynes/cm "minimal fouling" range as described by Dexter.

(3) In all graphs, except the 15 count analysis by dissection scope, these two substances experienced a relatively low degree of fouling.

(4) The general shape of each graph, with the exception of the same 15 count dissection scope analysis, was the same "backward N" form as shown by Baier.

(5) The general trend of fouling in relation to substrate surface energy, as described in both Baier and Dexter was also shown in this analysis, with the exception of the data taken on the 15 count dissection scope analysis.

(6) The fouling/surface energy relationship based on the dissection microscope 15 count data is contrary to both the other relationships established and graphed in this analysis, and the relationships established by Baier and Dexter.

Analysis of the two "after" HBS exposure graphs produced the following observations:

(1) The general form of each graph was a "W" in shape.

(2) Minimal fouling organism retention (or maximum fouling organism "release") was experienced by substrates having surface energies in the minimal fouling range of 22 to 24 dynes/cm described by Dexter (1979), and by those having surface energies in the mid 30's.

(3) Maximum fouling organism retention was experienced by substrates with surface energies below 22, between 27 and 30, and greater than 37 dynes/cm.

5. Analysis of Initial Fouling Organisms Using the SEM

During the Scanning Electron Microscope (SEM) analysis of the "before" and "after" HBS exposure samples, photographs were also made of typical, distinctive, or important fouling organisms. The majority of the matter present on the substrates was a mass of indistinguishable, mixed organisms and organic matter. Within this matter, however, were a few very distinguishable organisms, particularly species of settled phytoplankton, or periphyton, and species of protozoans. Shown in plates (15) through (20) of Appendix H are examples of these organisms.

Upon close analysis of the substrate photographs it was discovered that one particular organism seemed to be conspicuously present in samples of both "before" and "after" HBS testing. This organism was first noticed on the Hycar "after" photograph (plate (10)) where it constituted approximately 95 percent of the retained fouling matter. Continued analysis found these creatures to be present most

abundantly on Hycar, Teflon and Geon , but also, to some degree, on the rest of the substrates as well. The organisms or its secreted "shells" were in the form of roughly nemispheric mounds, 10 to 15 microns in diameter. Because of the "fluffy", white appearance of this structure, these structures were initially referred to as "sheep" until an identification of their correct name could be made. The distinctive structure which separated these "sheep" from the surrounding mixture of organic material, was a distinctive, 1 to 1.2 micron round hole, located off-center of the main structure. Detailed photographs of these fouling structures are shown in plates (21) through (24) of Appendix H.

To facilitate identification of the "sheep", X-ray scans of the nemispheric "shells" were made using a Tracor Northern NS-880 analyzer. This elemental scan showed the organisms to be composed primarily of, in order of greatest concentration; Manganese (Mn), Silicon (Si), Calcium (Ca), and Zinc (Zn). This information presented an interesting situation, because initial fouling has traditionally been considered an organic process; however, these "shells" were found to be composed largely of two heavy, inorganic metals; Manganese and Zinc. It was thought that these organisms may have combined elements existing in the surrounding environment, such as these metals, with a secretion from their own bodies, such as the carbonate ion, to create their shell or "test". Using the descriptions, drawings, and photographs presented in sources such as: Jahn (1949), Ray (1959), Grif-

fith (1961), Green (1971), Bick (1972), and Sieburth (1975), and through personal interviews with Dr. Gene Fischer and Mr. Jack Liberatori of NSRDC, an attempt was made to identify this organism. The "sheep" were initially thought to be an amoeba of the species Testacea, which, according to Jahn (1949) and Sieburth (1975), is an organism that colonizes surfaces, and which contains itself in a shell or "house" having a size, shape, and hole roughly equivalent to that observed in this analysis. This identification was found to be incorrect, however, when further investigation of the Testacea discovered that they are motile organisms which do not fully attach to the surface colonized. Since the organisms under investigation remained firmly secured to the substrate surface during extensive HBS testing, while the protozoans and bacterium fastened to the surface by mucopolysaccharide "glue" were removed, it was believed that the "sheep" must have been extremely firmly attached to the surface before HBS testing. Current theories on the identity of these fouling organisms are as follows:

(1) A sessile microscopic ameboid species, possibly related to the Testaceans.

(2) An initial stage of a Coralline Algae or an encrusting bryozoan which colonizes the substrate surface by secreting a test of surrounding environmental materials in the manner previously described.

(3) An inorganic structure which is a sedimentary product of the surrounding environment. The hole being caused by a rupture in the outer casing during evaporation processing.

C. Experiment Three

1. Analysis of Bacterial Transfer in Organically Enriched Artificial Sea water Solutions

The results of the bacterial transfer tests conducted using the solutions listed in Tables (5) through (10) are listed in Tables (18) through (23). Based on these results, the following summary was made:

(1) All three batches of the artificial sea water solution, created from the formula listed in Table (4), were found to support life, and therefore promote bacterial transfer in initial (trial #1) testing.

(2) Bacteria would not transfer, or transfer only slightly, through pure samples of artificial sea water solution during secondary (trial #2) testing.

(3) Bacteria would initially grow and transfer in initial tests (trial #1) of distilled water, but their degree of transfer was greatly reduced in secondary (trial #2) tests.

(4) Protein solutions, namely Peptone and Yeast, and sugar solutions, particularly, Sucrose and Glucose, facilitated bacterial transfer more readily that vitamin solutions.

(5) Solutions experiencing only slight degrees of transfer generally exhibited coccoid (round) species of bacteria, with bacillus (rod-shaped) species occurring only in solutions supporting heavier degrees of transfer.

(6) Only coccoid bacteria were found in vitamin solutions, especially the Lysozyme solution.

TABLE (18) - Results of Protein Analysis (Trial #1)

Solution	Degree of Trans. (*)
Severn River Water	4
Severn River Water (sterile)	4
artificial sea water (sterile)	1
artificial sea water (1/2 sal.) (sterile)	1
artificial sea water (1/2 sal.) + .005 % Peptone	3
artificial sea water (1/2 sal.) + .05 % Peptone	4
artificial sea water (1/2 sal.) + .005 % Yeast	4
artificial sea water (1/2 sal.) + .05 % Yeast	4

TABLE (19) - Results of Protein Analysis (Trial #2)

Solution	Degree of Trans. (*)
Severn River Water (sterile)	2
distilled water (sterile)	1
artificial sea water (1/2 sal.) (sterile)	Ø
artificial sea water (1/2 sal.) + .005 % Peptone	2
artificial sea water (1/2 sal.) + .05 % Peptone	4
artificial sea water (1/2 sal.) + .005 % Yeast	3
artificial sea water (1/2 sal.) + .05 % Yeast	4

* Degree of Fouling Key:
- Ø - no bacteria
- 1 - few single bacteria
- 2 - many single bacteria
- 3 - few bacterial colonies
- 4 - many bacterial colonies

TABLE (29) - Results of Sugar Analysis (Trial #1)

Solution	Degree of Trans. (*)
distilled water (sterile)	3
artificial sea water (1/2 sal.) (sterile)	1
artificial sea water (1/2 sal.) + .005 % Sucrose	4
artificial sea water (1/2 sal.) + .005 % Galactose	2
artificial sea water (1/2 sal.) + .005 % Glucose	2
artificial sea water (1/2 sal.) + .005 % Lactose	2

TABLE (21) - Results of Sugar Analysis (Trial #2)

Solution	Degree of Trans. (*)
distilled water (sterile)	1
artificial sea water (1/2 sal.) (sterile)	Ø
artificial sea water (1/2 sal.) + .005 % Sucrose	2
artificial sea water (1/2 sal.) + .005 % Galactose	2
artificial sea water (1/2 sal.) + .005 % Glucose	3
artificial sea water (1/2 sal.) + .005 % Lactose	2

* Degree of Fouling Key:
Ø - no bacteria
1 - few single bacteria
2 - many single bacteria
3 - few bacterial colonies
4 - many bacterial colonies

TABLE (22) - Results of Vitamin Analysis (Trial #1)

Solution	Degree of Trans. (*)
distilled water (sterile)	Ø
artificial sea water (1/2 sal.) (sterile)	1
artificial sea water (1/2 sal.) + .005 % Vitamin B	1
artificial sea water (1/2 sal.) + .005 % Lysozyme	2
artificial sea water (1/2 sal.) + .005 % Niacin	1
artificial sea water (1/2 sal.) + .005 % Vitamin B6	1

TABLE (23) - Results of Vitamin Analysis (Trial #2)

Solution	Degree of Trans. (*)
distilled water (sterile)	Ø
artificial sea water (1/2 sal.) (sterile)	Ø
artificial sea water (1/2 sal.) + .005 % Vitamin B	1
artificial sea water (1/2 sal.) + .005 % Lysozyme	3
artificial sea water (1/2 sal.) + .005 % Niacin	1
artificial sea water (1/2 sal.) + .005 % Vitamin B6	1

* Degree of Fouling Key:
Ø - no bacteria
1 - few single bacteria
2 - many single bacteria
3 - few bacterial colonies
4 - many bacterial colonies

2. Analysis of Initial Bacterial Colonization of Glass Slide Sections

Shown in Appendix I, plates (25) through (33), are sequential photographs of initial bacterial colonization of submerged sections of glass microscope slides. The submergence solution used was 100 ml of artificial sea water (1/2 salinity), enriched with .05 % Peptone protein, and containing periphytic bacteria originating from the .05 peptone solution tested in trial #2 of the protein analysis. The solution was allowed to cure for 24 hours before immersion of the slide sections.

Analysis of the photographs of fouling bacteria revealed small, circular organisms resembling the previously mentioned "sheep" attached to the glass slide surfaces. Because of these organisms' size of less than 1 micron in diameter, clear close-up photographs were not possible. An elemental x-ray scan showed these organisms to be composed almost exclusively of Calcium and Silicon. It was suggested that these these organisms might be an initial life form of the "sheep" discovered in experiment 2. In order to test this, a 1 cm by 1 cm square of Hycar, which had proven to be the "best" substance for "sheep" colonization, was submerged for 144 hours in the .05 percent Peptone solution described earlier. SEM analysis of the sample showed many rod-shaped bacillus and round coccus fouling bacteria surrounded by much organic material, but none of the "sheep-like" organisms. A sample photograph of this growth is shown in Appendix I, Figure (34).

V. Discussion, Conclusions, and Recommendations

A. Experiment One

1. Water Quality

The observations presented earlier outlined several trends concerning tne water variables analyzed, namely salinity, conductivity, oxygen content, and temperature, as they were observed over the two week sampling period. Salinity and conductivity, which often reflect each other because the concentration of salt ions making up a saline solution directly affect that liquid's conductivity, appeared to form a gradient typical of an estuarine system such as the Severn River (Green, 1971). Stations 2 and 3, located at the river's mouth, demonstrated 70 and 80 percent of tne highest values for salinity and conductivity, respectively. Stations 6 and 7, located at the river's head waters, experienced all of the lowest or second lowest values for both salinity and conductivity. This information indicated that a sufficient gradient in both of these variables had been established to warrant their use in comparative research.

Less of a gradient was determined to exist in oxygen content, as several different stations experienced high and low values over the sampling period. This was expected due to the variety of oxygen sources, such as organic matter, creek or stream inflow, and tidal cycling which may affect the oxygen content in sea water solution. A general trend

shown by the graphical analysis was a low oxygen content occurring on the first sampling day followed by a steady increase in the following days. This could have been the result of the previously mentioned thunderstorm, occurring on 19 October, 1982, stirring the water sufficiently to cause a noticeable increase in its oxygen content. Generally, the highest oxygen contents were found at stations located in open areas, namely 5 and 6. This was probably due to strong, unobstructed tidal flushing and wind mixing which removed oxygen depleted water, and replaced it with oxygen rich water. Low oxygen values occurred in areas located farthest from shore and in relatively deep water, such as stations 2 and 3. This is probably due to less marine plant life which exude oxygen in their normal metabolic activity being present at these sites than at sites located in shallower water.

Temperature of the river water sampled was subject to many fluctuations. These variations could been the results of weather, tide, sampling time, or some combination of these factors. The general trend was a steady decrease during initial sampling days, with a low occurring on the tenth day, followed by an increasing trend on the fourteenth day. This initial downward trend may also have been caused by the thunderstorm which occurred at the beginning of the sampling period. During the mixing process caused by its heavy winds, the storm may have brought deep, cool water to the surface where the slide boxes were located.

2. Fouling, and Fouling as Related to Water Quality

Since the information on fouling vs. time, presented in appendix B, concerns relative fouling between the 7 stations on each sampling day, only comparative trends were shown. Some locations, such as stations 2 and 5, remained relatively constant throughout the analysis period, while others, particularly stations 3 and 7 were subject to more fluctuations. Since the glass substrate slides, sampling boxes, and depth of submergence was equivalent for each station, these variations must have been the result of changes or occurrences that existed in the environment surrounding the sampling stations.

The comparative analysis between water variables and relative fouling, graphically depicted in appendix C, shows no direct linear relationship between these quantities. However, according to Green (1971), gradual changes in any of these variables could result in existing organisms adapting to life in the new environment, whereas rapid changes of similar magnitude could prove fatal. Salts and salt ions are necessary for normal metabolic activities of many organisms. Natural protective systems within an organisms body can overcome slight salinity variations, but drastic increases in salinity can cause osmotic imbalances to permeable membranes within the organisms. This may result in severe tissue damage, and subsequent destruction of the organism (Meglitsch, 1967). However, estuarine organisms, such as those found in the Severn River, are generally

tolerant to a wide range of osmotic pressures. Oxygen is necessary for metabolic activities in all aerobic (oxygen-using) organisms. Some microorganisms living in waters gradually being depleted of oxygen, from excessive biologic activity, pollution, or other similar processes, may change to anaerobic respiratory methods in order to compensate, however, rapid decreases in water oxygen content may result in the destruction of all but the already existing anaerobic life forms (Woods Hole, 1952). Temperature may also affect reproduction and growth rates of many fouling organisms. For example, in one study the barnacle Balanus balanoides, varied as much as 95.6 percent in growth and maturation rates between temperate and tropical waters (Woods Hole, 1952).

In experiment one, the highest degrees of relative fouling generally occurred at stations where the water was relatively low in salinity (4 to 6 ppt), high in oxygen content (> 10 ppm), and warm (15 to 18 degrees C). However, it should be noted that only mild fluctuations of the variables analyzed occurred over the 2 week analysis period. This may have been due to the sampling period being too brief to cover greater changes, or due to the relative stability of the Severn River during the period analyzed.

One possible explanation for the lack of a direct relationship between relative fouling and the water characteristics analyzed is that water variables not only affect fouling organisms, but also each other. Green (1971) stated

that solubility of oxygen in water is influenced by both salinity and temperature, while temperature alone can influence the amount of dissolved solids (i.e. salts) that a solution can support. It is therefore possible that the reason for the complex trends of relative fouling discovered were not due to fluctuations of any of these water variables alone, but a combination of some or all of them. Another possible explanation is that some other water characteristic, such as depth of water, tidal fluctuations, or dissolved gasses, may have caused or influenced the results obtained.

3. Organism Analysis

A comparison between the organisms observed growing on the submerged glass slides in experiment one and the previously listed chronologic succession of fouling, as described by Cologer (1979) and Fischer (1982), shows that, in the two week sampling period, the observed growth had progressed through the third stage of protozoans. All of the fouling organisms discovered were common microscopic plants and animals found in the Chesapeake Bay area. The fouling succession discussed in the literature was observed, however, in some cases, such as stations 2 and 3, one stage, namely protozoans, seemed to dominate at the expense of the other stages such as diatoms and algae.

AD-A134 290 SURFACE ENERGIES AND CHEMICAL ANALYSIS OF THE INITIAL STAGES OF MARINE MICROBIOLOGICAL FOULING(U) NAVAL ACADEMY ANNAPOLIS MD M J OLSON 20 JUN 83 USNA-TSPR-127 2/3

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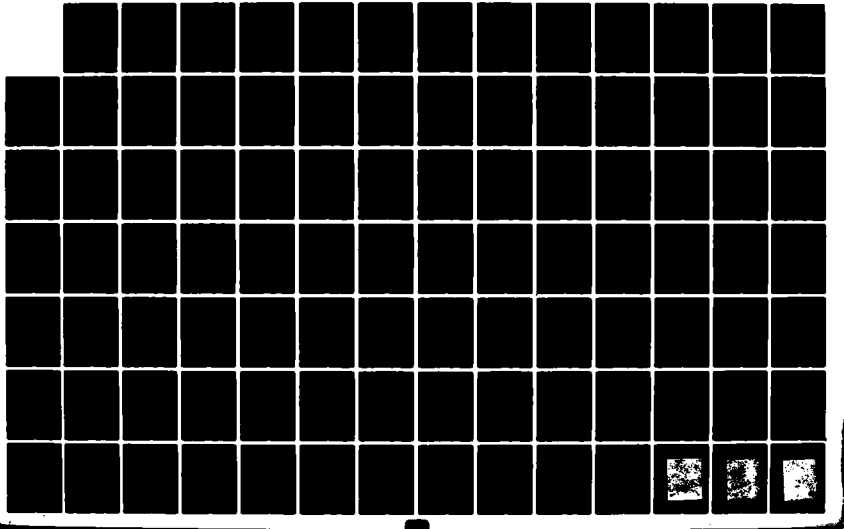

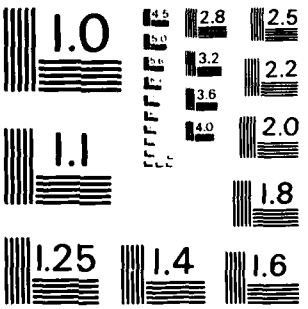

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4. Recommendations for Further Research

More studies of the effects that water characteristics and composition have on species types, reproductive rates, and metabolic activities of fouling organisms is warranted, because a good understanding of these relationships could result in better prediction of fouling for specific areas, or as a base for methods to prevent or control fouling. Subsequent studies should cover a wide range of variables including such factors as dissolved gasses, water movements, and alkalinity. Furthermore, a longer sampling period of 6 months to a year, beginning in the early spring and running through late summer or fall, would produce more significant changes in water variables, rapidity of fouling, and variations in species of fouling organisms.

B. Experiment Two

1. Surface Energy Determination

The topic of surface energies and their relationship to biological fouling is a relatively new field of investigation. There are still many aspects of this type of analysis, such as specific methods, standardized values, and ranges of error deviation, which remain unknown or undefined. The contact angle goniometer method employed in this analysis, is currently one of the more common methods in surface energy determination because of its relative ease of procedure and accuracy of results. It is not always necessary to use as large a sample of contact angle liquids as was used in this analysis. Becka (1981) used only samples of triple distilled water, reagent grade glycerol, methylene iodide, and purified n-hexadecane in her determinations. Since a relatively large range of surface energies was to be determined in experiment two, it was believed that more liquids would provide more statistically reliable data. Furthermore, the use of several liquids reduced the risk of contamination of one or two liquids having a significant effect on the experimental results.

As mentioned earlier in the results of experiment two, a problem that arises during surface energy determination is that, certain materials, though having the same name and general composition, may vary significantly in specific composition between different manufacturers, or even different batches made by the same manufacturer (Griffith, 1983).

These differences can cause surface energy variations between these substances of as much as 10 dynes/cm. Some investigators claim that surface energies can also differ due to surface texture. Both Becka (1981) and Dexter (1977) found differences of as much as 8 dynes/cm between surface energies of polished and unpolished substances. On the otner hand, contamination during polishing, and increased uncertanties in contact angle measurements on rough surfaces may also have been responsible for these findings.

In experiment two, all of the surface energy values used for relative fouling comparison fell within +/- 3 dynes/cm of the already established surface energy value or range of values established for that substance. This relatively small margin of error was most likely caused by the variations in composition or texture listed above. Of the substrates analyzed in this experiment, only the Hycar and Silastic rubbers were of concern due to possible variations in their composition, while samples of Teflon and Polypropylene were of concern due to variations in their surface texture. The graphs shown in appendix D illustrate how the different substrates varied in the linearity of their data points and the "slope" of the line "fitted" through these points. For example, the data points for Teflon (Figure (68)) are much more linear in nature, with a steeper slope than those for Geon (Figure (73)), whose points seem to bunch up at the top of the graph. The more linear the points,in general, the more accurate the data. This can be

seen in the results, where the determined surface energy for Teflon varied from the established value by less than 1 dyne/cm, while that determined for Geon varied by almost 3 dynes/cm.

2. Analysis of Surface Energy vs. Fouling

The two methods of fouling analysis used in this experiment, namely, colony counts made with the dissection microscope and relative ranking of Scanning Electron Microscope (SEM) photographs, differed in basic theory. Because the former was an actual quantitative analysis which produced specific results, while the latter was a qualitative analysis which, similarly to the relative ranking used in experiment one, produced data on a substrate which was meaningful only when when compared to similar data on the other substrates. A current problem in fouling research is placing specific, quantitative numbers on amounts of fouling observed. The method of counting colonies of fouling organisms used in this study provides good data if the number of counts made is sufficiently large, however, this method is a long and tedious process, especially when several samples are to be analyzed. Another method for quantitative analysis frequently employed measures the amount of electron transmissions passed through a fouled sample and compares it to transmissions passed through the same sample before fouling (Baier, 1977). This method provides specific, numeric values which can be used to determine the amount of fouling present, but is only applicable for transparent or highly

translucent samples. It was thus inapplicable for use in this analysis, since most substrates were opaque in nature.

Graphs of surface energy vs. fouling or relative fouling (i.e. "before" HBS testing) displayed in appendices E and G show a significant resemblance to the relationship described by Baier (1977) and shown in Figure (3). The general shapes of the graphs from experiment two are, with one exception, the same "backward N" form as shown in Baier, with low fouling values occurring between 22 and 24 dynes/cm and above 39 dynes/cm, and with high fouling values occurring in the range between the upper 20's and lower 30's. As previously mentioned, to say that something is a "low" fouler is, in itself a relative statement, as "low" fouling does not mean "no" fouling, but only a slower rate of fouling (Fischer, 1982).

The exception to these graphs is the one based on the data collected by the 15 count sampling shown in Figure (76). As mentioned in the results, these data are contrary, not only to the fouling trends established through earlier research, but also to those found to exist in the other runs of this experiment. The major points of inconsistency are those assigned to teflon, showing very low fouling, and those assigned to Dimethyl Polysiloxane and Geon, showing very high fouling. This inconsistent, irregular data may be the result of errors such as contamination of substrates by skin oils, or roughness of the specific samples which made them more hospitable to bacterial attachment.

3. Analysis of Surface Energy vs. Fouling Release

The concept of relating the surface energy of a substance to the tenacity with which fouling organisms will attach to it is a new field of research. Again, the problem arises of trying to convert the observations made into specific, quantitative numbers which can be used for more than purely a comparative analysis. The methods of colony counting and relative ranking employed in this analysis provided both quantitative and qualitative results, but lacked the precision of methods such as the electron transmission method mentioned earlier. The graphical results of these analyses, shown in Figures (78) and (80) of appendices E and G, respectively, show a similar pattern. Low retention of organisms occurred not only in the 22 to 24 dynes/cm minimal fouling range, but also in a higher range near 35 or 36 dynes/cm. This is very significant, because substances found in these ranges might prove to be much more easy to clean of fouling organisms than substances falling outside of these ranges.

4. Analysis of Fouling Organisms Encountered.

As previously discussed, concept of combating fouling through an understanding of its processes and components constituted the main rationale for this analysis. A substance classified as a "low" fouler or good "retainer" of fouling organisms may be so only because some aspect of it has an influence on one or more stages in the fouling succession. Such may be the case with the "sheep" encountered. These structures are important as foulers for two main reasons. First, because they were a major initial fouling constituent which was appeared on all of the substrates analyzed. Second, because these structures had a high retention on substrates exposed to HBS testing. These organisms remained attached to the substrate surface while other organic materials, including initial fouling bacteria were removed. This means that either: (1) the "sheep" grew upon the bacterial layer and subsequently penetrated through it, into the actual substrate surface, or (2) the "sheep" attached to the substrate *before or concurrently* with the initial fouling bacteria. While either of these theories are possible, the latter is more intriguing as bacteria have long been considered the initial living stage in the fouling succession. If these "sheep", in one form or another, do attach before the bacteria, then the established fouling succession must be revised.

Another characteristic about these organisms which makes them both interesting and puzzling is their elemental

composition of significant amounts of Manganese and Zinc. While both of these heavy metals are contained in the bodies of many living things, neither constitutes a major part of fouling organisms known. Bacterial fouling, as currently envisioned, consists of the deposition of mucopolysaccharides as one of its initial layers. The presence of "sheep" suggests that a layer of inorganic material with a great deal of adherence is also an important stage in the fouling succession. One possible explanation, as discussed in the results section, is the ability of some organisms to create a shell or "test" by combining excretions from their bodies, particularly the carbonate ion, with certain cations existing in the surrounding environment (Meglitsch, 1967). According to Dana (1944) and Berry and Brian (1959), common inorganic materials exist which are combinations of the four major constituents found in "sheep" and the carbonate ion. They are:

Rhodochrosite	$MnCO_3$
Smithsonite	$ZnCO_3$
Calcite	$CaCO_3$
Rhodonite	$Mn(SiO_3)$

Fischer (1983) stated that an overabundance of heavy metals in estuarine systems is common, due to excessive water traffic and industrial wastes. The presence of Zinc in the the water could be a result of galvanization of hull surfaces or leaching by zinc containing paints. The presence of Manganese in quantities sufficient enough to constitute its being the major component in "sheep" is less easily

explained as little commercial or industrial use which would cause pollution of an estuarine system currently exists (Fischer, 1983). These findings are sufficiently unusual and possibly significant so as to warrant further research.

5. Recommendations for Further Research

The concept of surface energies as related to both degree of fouling experienced and amount of fouling release is a new and potentially productive field. Further research is necessary to more accurately determine surface energy values for use as references in fouling comparisons. More precise determinations of surface energies and better quantitative methods of measuring degrees of fouling and organism retention must be developed. The surface energy ranges supporting low fouling and low retention should be extensively investigated because it is possible that capitalization on these effects could produce better antifoulants or methods of cleaning which might lead to substantial savings in both time and money. Further investigation of the the stages which make up the fouling succession, including a positive identification of the "sheep", is warranted because control and prevention of marine fouling can only come through an understanding of its components and processes.

C. Experiment Three

1. Artificial Sea Water Solution

The creation of a solution which will model the properties of sea water is a difficult process. This is because salts, and other inorganic components which make up natural sea water will vary in number and concentration based on geographical and seasonal differences (Woods Hole, 1952). Also, other ingredients existing as "trace elements" in salt water solutions, such as sulfur, metallic ions, and dissolved gasses may also warrant consideration, due to the effect that they have on existing marine life. The Lyman and Flemming (1934) artificial sea water solution created for use in experiment three was a generalized solution containing only inorganic salts. No considerations were made concerning trace elements in this solution, because of the variability of these substances in naturally occurring sea water and because of difficulties in weighing and controlling such small amounts of materials. Care was taken during the creation of solution batches to ensure that sterility of materials and and exactness of ingredient measurements was maintained in order to prevent erroneous data values caused by solution contamination. The solution created for this analysis was cut to 1/2 of its original salinity in order to more closely match the salinity of the the Severn River. This solution proved to be good for analysis as it would support life and bacterial transfer after enrichment with only slight amounts of organic material. Initial tests used

samples of both the original stock solution and this half-cut solution, but after bacterial transfer analysis showed both these solutions to react basically the same, only samples of the half-cut solution were used in latter testing.

2. Analysis of Bacterial Transfer Solutions

The basic analytic procedures used in experiment three were based on the research done by Clop' (1981), in which samples of sterile Severn River water ere organically enriched, implanted with fouling bacter and any subsequent bacterial transfers noted. Artific' ea water was used instead of Severn River water in this analysis so that the exact composition of the solution being enriched and analyzed would be known, in an attempt to make explanation of results, based on water composition, possible. Solutions of pure artificial sea water were found to promote bacterial transfer in initial (trial #1) tests probably because there was enough dissolved nutrient contained in the film on the "fouled" slide used for bacterial transfer to cause slight initial enrichment of the solution. Artificial sea water would not support life during secondary testing because these nutrients had been depleted from the solution by the fouling bacteria, leaving insufficient residual materials in the film surrounding the second "fouled" slide to enrich the trial # 2 solutions.

Distilled water proved to contain many fouling bacteria which would transfer during both initial and secondary testing. Since initial autoclave and milipore filter sterilization should have destroyed all bacteria existing in the original distilled water sample, it can be concluded there was some material or materials present in the distilled water which were not destroyed by sterilization and which would promote bacterial transfer. The distilled water used originated from the DW line system of the chemistry laboratories at the Naval Academy. It is possible that some materials were present in solution when this water was drawn from the tap. It is also possible that the nutrients contained in the water film surrounding the "fouled" slides were sufficient to support life in the distilled water over the test period. If this were true, then this material would be depleted by the bacteria present after a period of time, as was the case in the artificial sea water solutions. This would mean that the distilled water might promote bacterial transfer, but only for a limited time period.

Since all three groups of enriching agents, namely proteins, sugars, and vitamins, when added to artificial sea water produced solutions which would support and sustain life and bacterial transfer, it can be concluded that the initial layer of organic material mentioned earlier may be composed of any, or all of these compounds. All of these materials formed thin coatings over the submerged glass plates which were suitable for initial bacterial attachment

and growth. Of special note was the Lysozyme used in the vitamin analysis. This substance is not actually a vitamin, but an enzyme which destroys the cell walls of gram positive bacteria, yet the Lysozyme solution proved to have the highest degree of bacterial transfer in the both trials of the vitamin analysis. One possible explanation of this is that the concentration of Lysozyme in solution was so low that the bacteria were actually using it as a nutrient. Comparison of the degree of bacterial transfer found in Lysozyme solutions to those listed in Tables (17) and (18), found in initial protein (Peptone and Yeast) solutions of similar concentration, however, show that these initial solutions promoted a significantly greater degree of bacterial transfer than the Lysozyme solution. It is a more likely, then, that the reason that the Lysozyme solutions promoted a relatively large degree of bacterial transfer was that the elimination of the gram positive fouling bacteria reduced much of the nutrient competition of the remaining gram negative bacteria, allowing them to grow and reproduce more rapidly.

Also of note was the observation that solutions experiencing only mild degrees of bacterial transfer exhibited the coccoid (round) bacteria, while bacillus (rod-shaped) bacteria were only discovered, intermixed with coccoid species, in solutions promoting larger degrees of transfer. From this it can be concluded either that, in this analysis the initial fouling bacteria were coccoid

species, which created surfaces onto which the latter bacillus species attach, or that the materials used to enrich the solutions which produced mild bacterial transfers were of such a nature that they would only, or primarily support species of coccoid bacteria.

3. Analysis of SEM Photographs of Bacterial Fouling

The photographs shown in appendix I demonstrate the chronological colonization of bacteria on a submerged substrate, in this instance glass microscope slides. The transfer solution used for this analysis was artificial sea water enriched with .05 percent Peptone. This particular solution was chosen because it had proven to promote a large degree of transfer of both coccus and bacillus bacteria in initial tests. Initial photographs of slides after 12 and 24 hours of submergence show only a few scattered coccus and bacillus bacteria on the substrate surface. After 48 hours of submergence a mass of material can be seen forming around the bacteria (Plates (28) and (29)). This material is believed to be mucopolysaccharide glue which has been secreted by the bacteria in order to firmly attach themselves to the substrate surface. Later photographs of slides after longer periods of submergence show the development of colonies of both coccus and bacillus bacteria. These organisms continued to grow and reproduce as they colonized the surface until they had created the extensive colonies shown in plates (32) and (33).

Intermixed with the colonies of fouling bacteria found on the glass slides were the small organisms resembling the "sheep" described in experiment two. An elemental scan of these organisms showed them to lack the heavy metals of Manganese and Zinc found in the original "sheep". It is possible that, since the original organisms had been grown in Severn River water and the second, smaller organisms originated from an enriched artificial sea water solution, that this composition difference might have been due to the differences between the ingredients in the two "parent" solutions. The exact composition of the Severn River water in which the original "sheep" were grown was not known, but, as shown in Table (4), the artificial sea water solution in which the smaller organisms grew was lacking in both Manganese and Zinc. If the theory of how the "sheep" make their tests by combining certain existing cations with body excretions, such as the carbonate ion, is assumed to be true, then the reason why the organisms discovered in experiment three lacked both Manganese and Zinc, might have been that it was not available to them in solution. A flaw in this theory, however, was that the artificial sea water solution listed in Table (4) also did not contain Silicon, which was a major component in the smaller "sheep". The fact that no "sheep" of any noticeable form were discovered on the submerged Hycar sample as shown in plate (34), also made it questionable as to whether the two "sheep" organisms were actually different forms of the same organism. This

was because linear had proven to be the substrate most readily fouled by the original "large sheep". Based on this it was concluded that the small "sheep" discovered in experiment three were probably not the same organisms as those observed in experiment two. Instead, they were believed to be some form of microscopic organisms, possibly diatoms or bacteria, simply resembling the earlier organisms.

4. Recommendations for Further Research

A continued, specific analysis of the components which constitute the formation of the initial organic film and transfer of bacteria is warranted. Many organic nutrients and compounds may exist within a naturally occurrng sea water which will promote the growth of initial fouling organisms. Trace elements and dissolved gasses may also affect the rate or degree of bacterial transfer that a solution will promote by adding more materials necessary for bacterial growth.

More research is also warranted based on the two possible theories for why only coccus bacteria were observed in solutions experiencing minimal fouling. If the former of these was found to be true, then subsequent research might be performed to further breakdown the stages of bacterial fouling in the fouling succession in order to determine the chronological succession of the bacteria themselves. If the latter theory were proven true, then research might be done to look at the relationships existing between specific species of bacteria and specific compositions of initial

adsorbed organic layers. As mentioned earlier, an understanding of the initial stages of marine fouling is necessary in order to combat or control this complex, successive process. If the initial stages of the fouling succession, such as the microfouling stage, could effectively be deterred, latter stages of fouling could also be greatly reduced, making hull coatings last longer, extending the time necessary between dry docks, and saving the Navy operational time and money.

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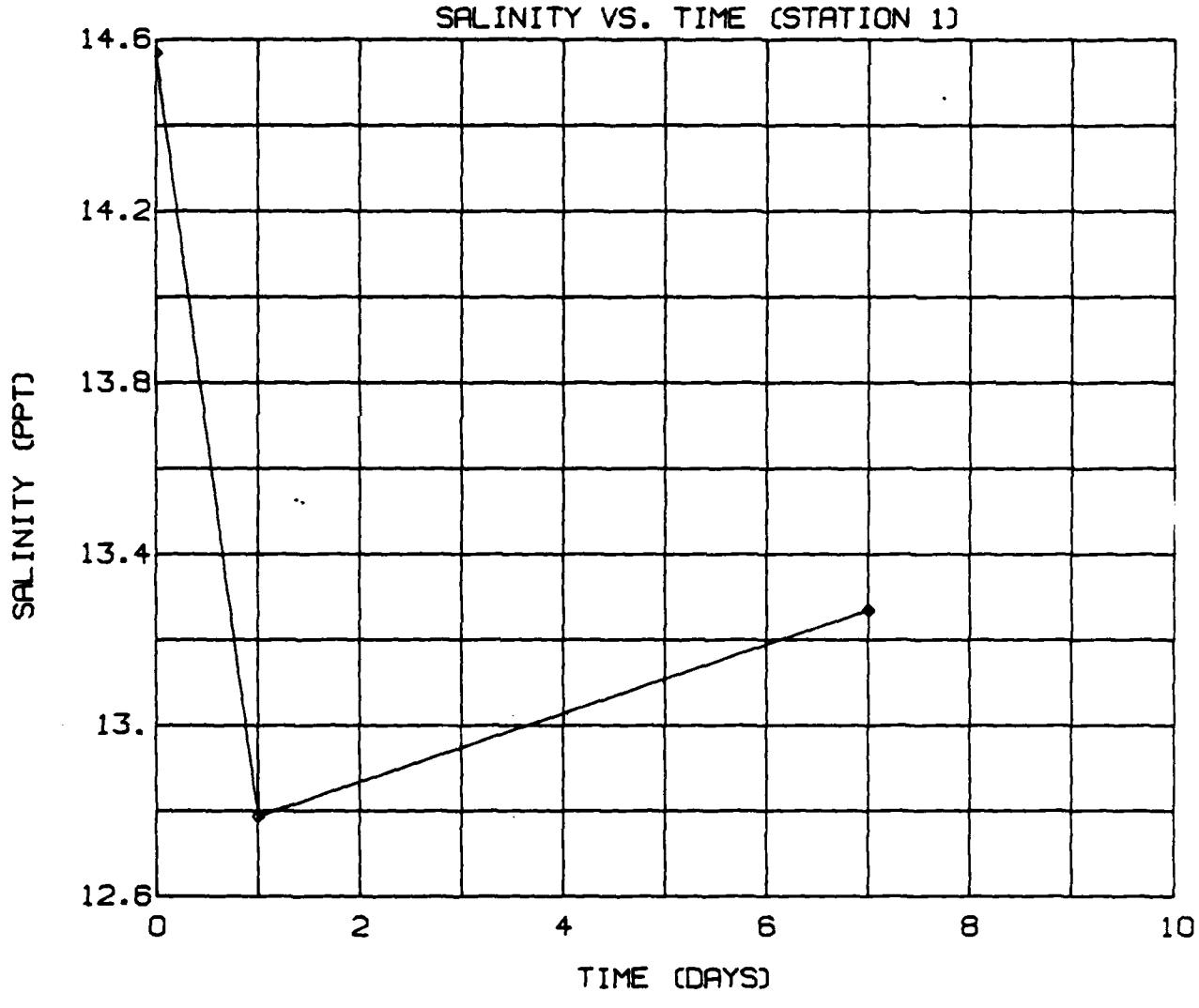
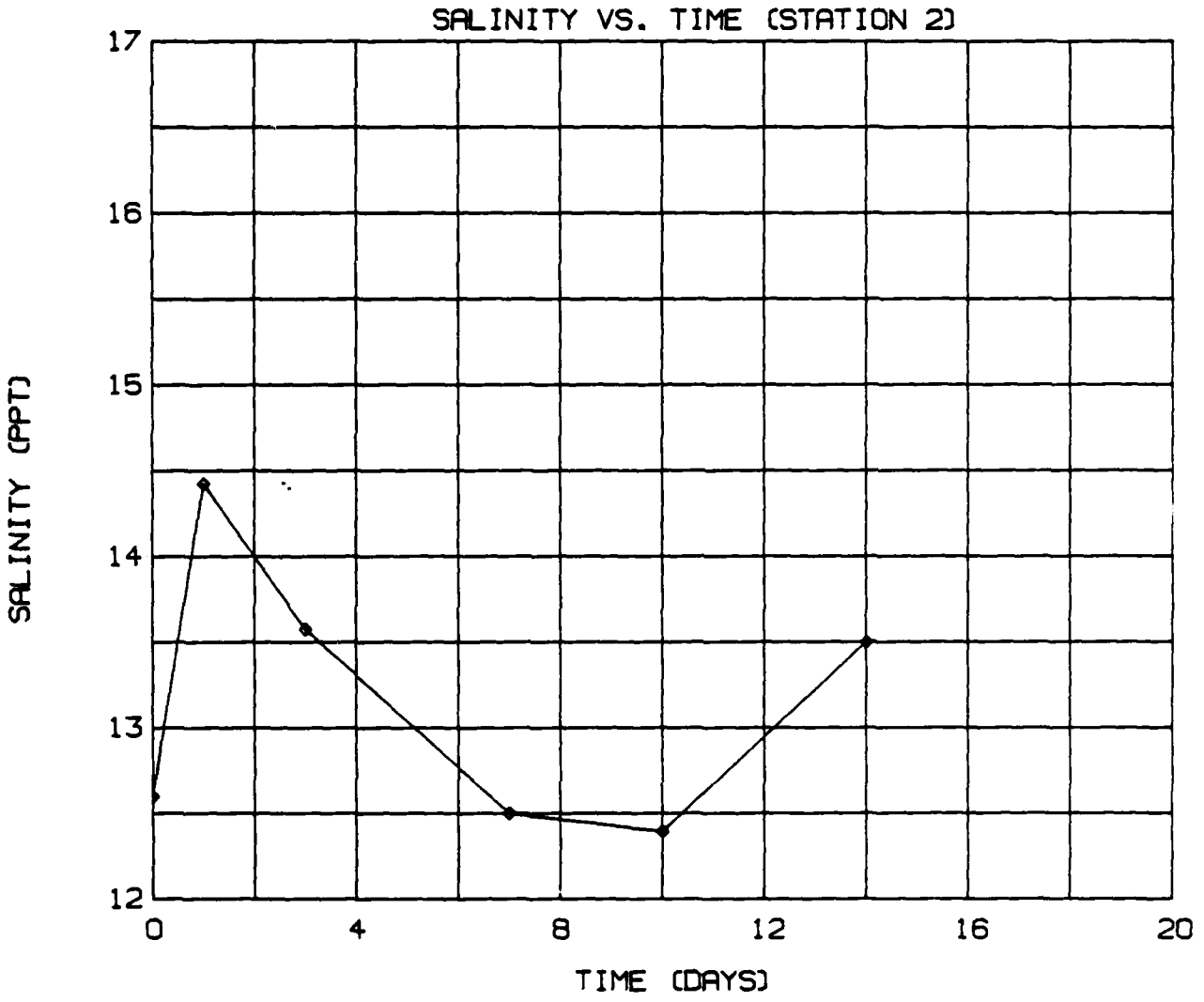
APPENDIX A - EXPERIMENT ONE:

GRAPHS OF WATER VARIABLES VS. TIME

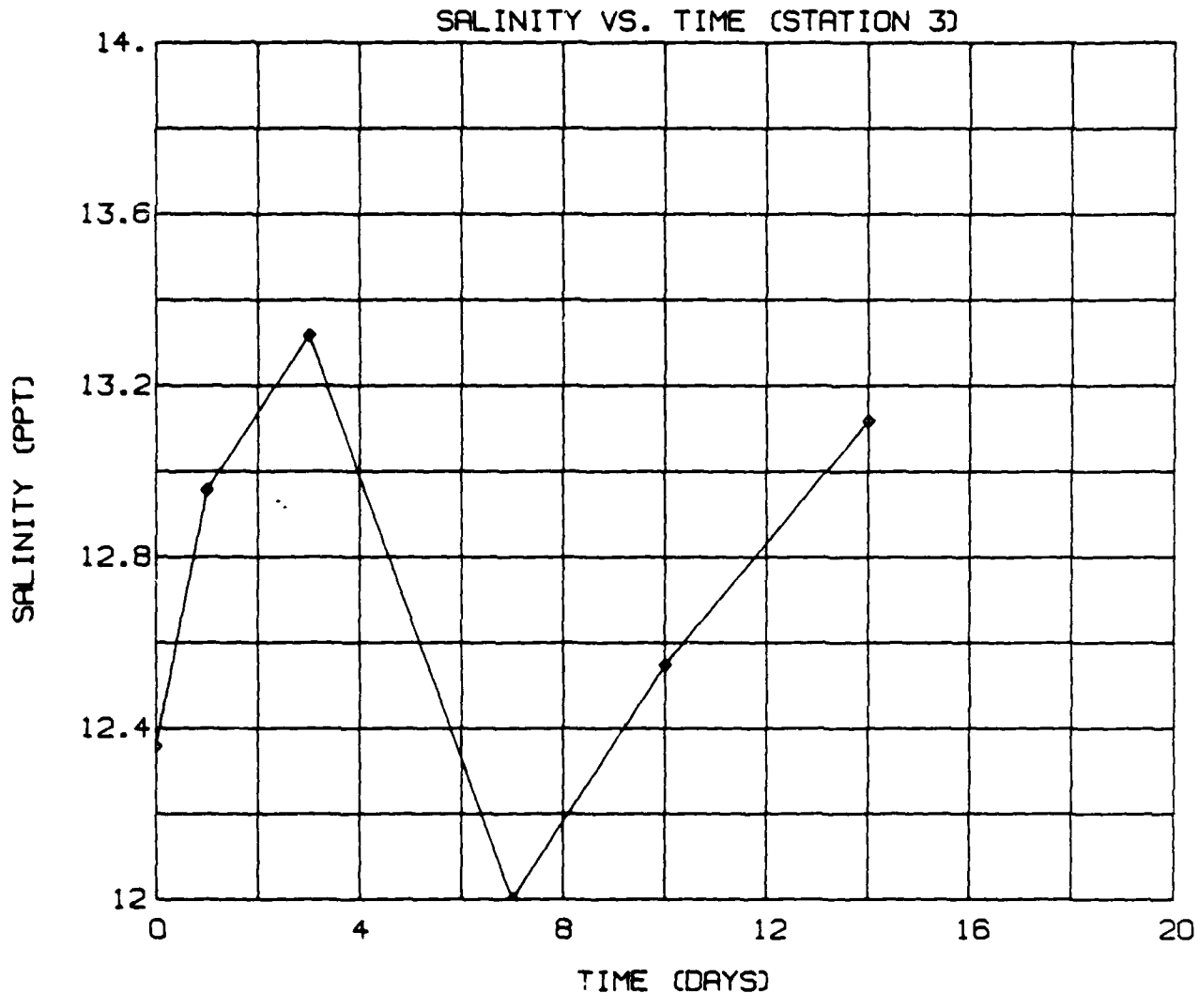
- FIGURE (13) -


SALINITY VS. TIME (STATION 1)
SALINITY (PPT)
14.6
14.2
13.8
13.4
13.
12.6
0
2
4
6
8
10
TIME (DAYS)

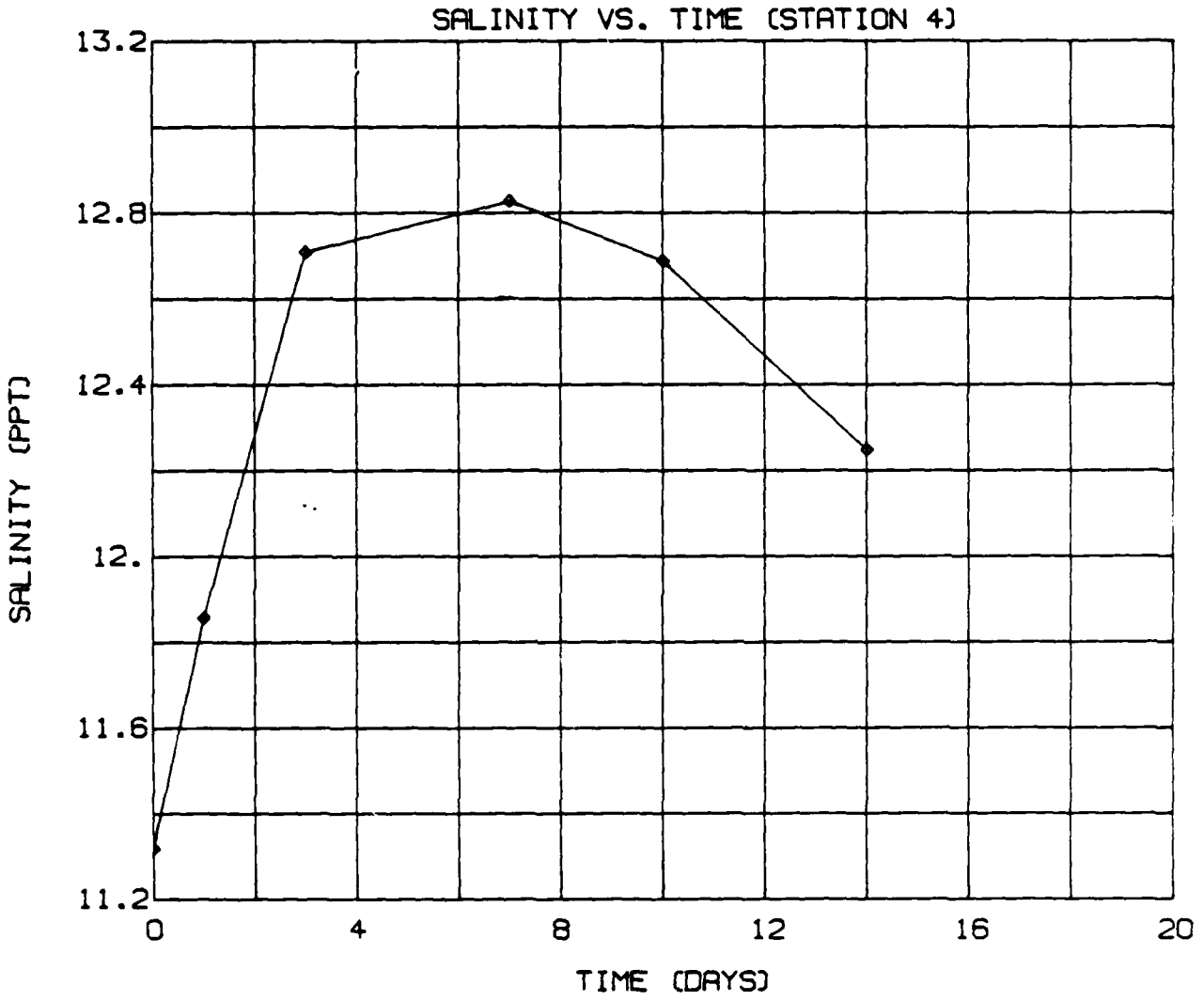
- FIGURE (14) -


SALINITY VS. TIME (STATION 2)
SALINITY (PPT)
17
16
15
14
13
12
0
4
8
12
16
20
TIME (DAYS)

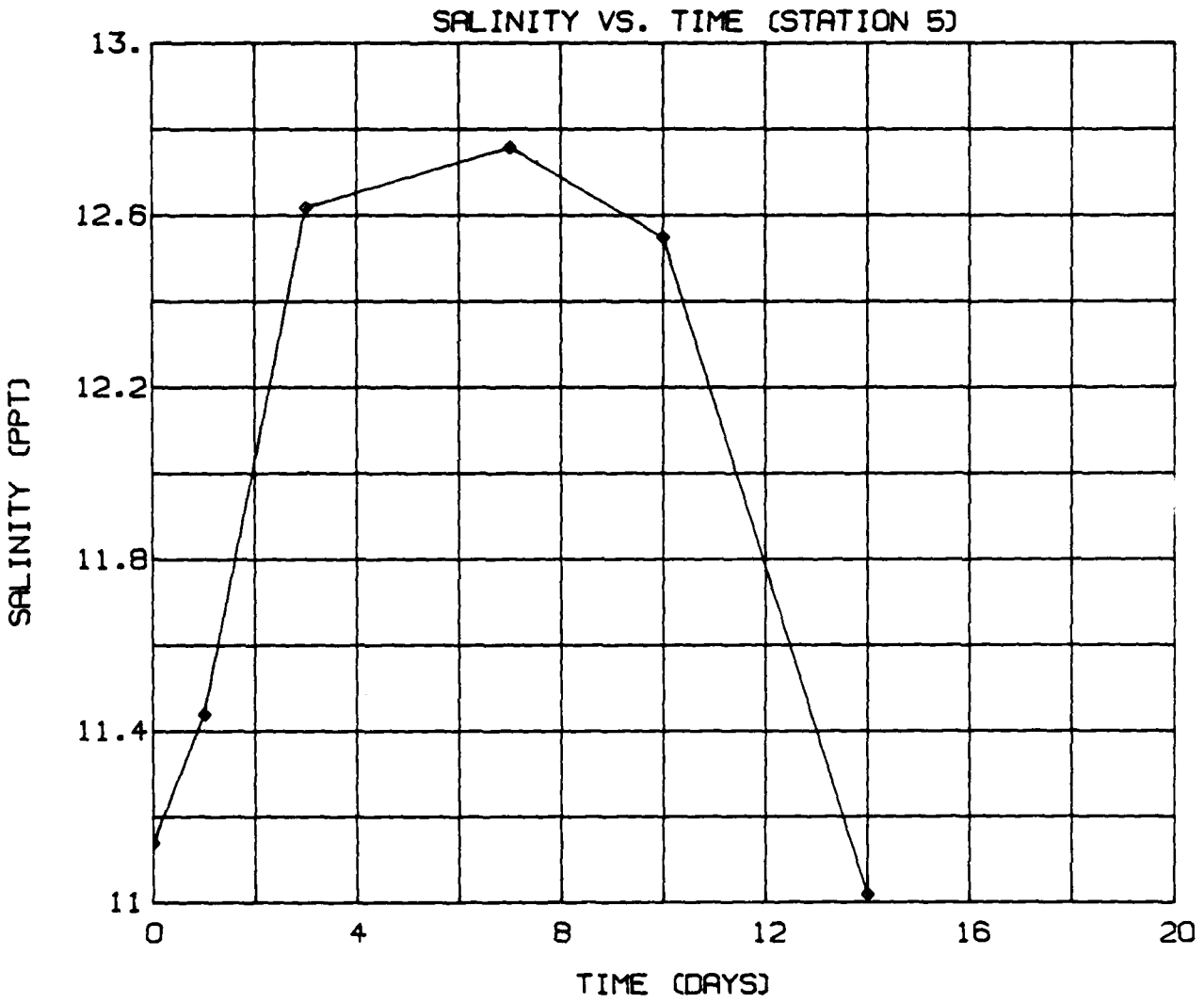
- FIGURE (15) -


SALINITY VS. TIME (STATION 3)
14.
13.6
13.2
12.8
12.4
12
SALINITY (PPT)
0
4
8
12
16
20
TIME (DAYS)

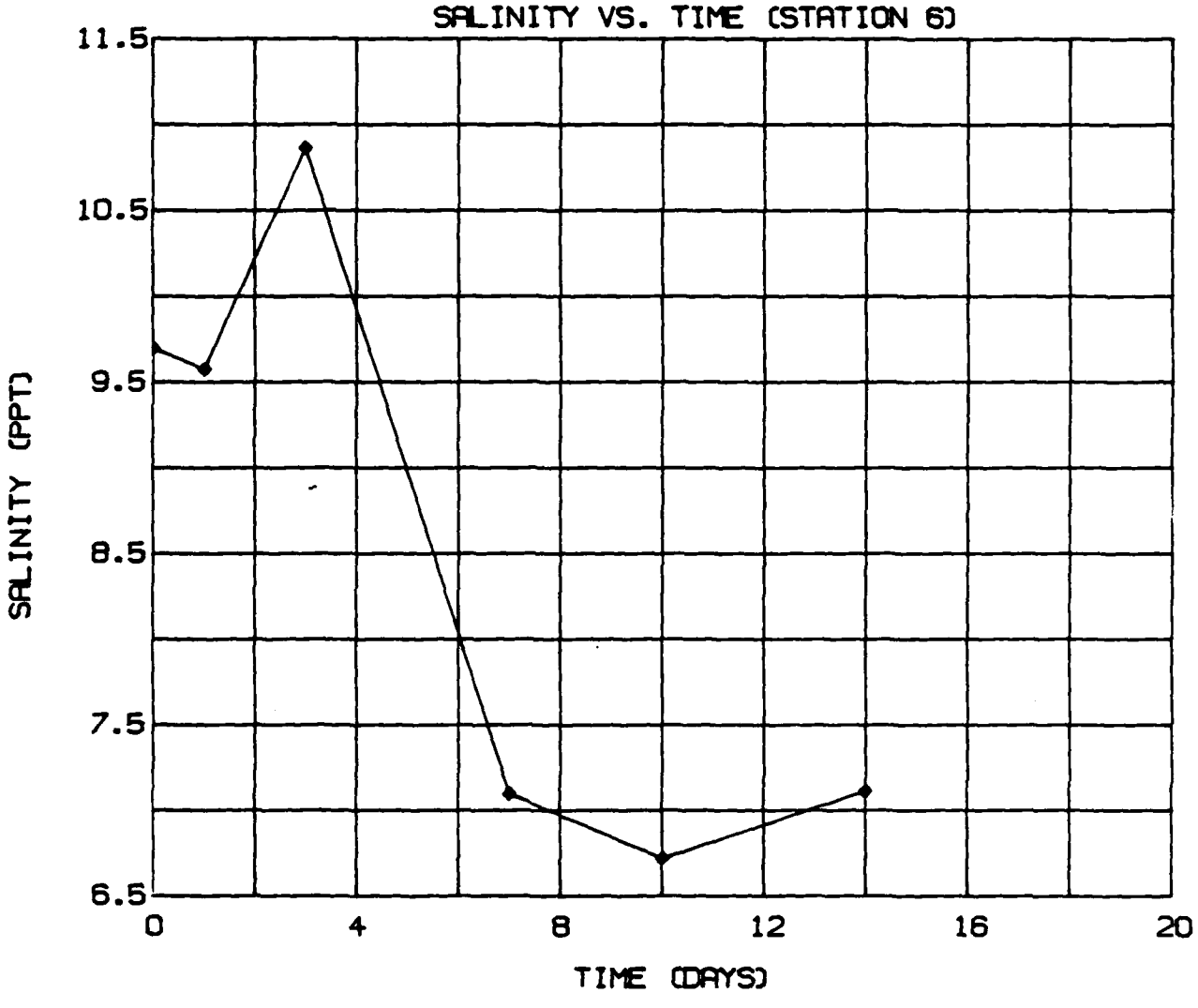
- FIGURE (16) -


SALINITY VS. TIME (STATION 4)
13.2
12.8
12.4
12.
11.6
11.2
SALINITY (PPT)
0
4
8
12
16
20
TIME (DAYS)

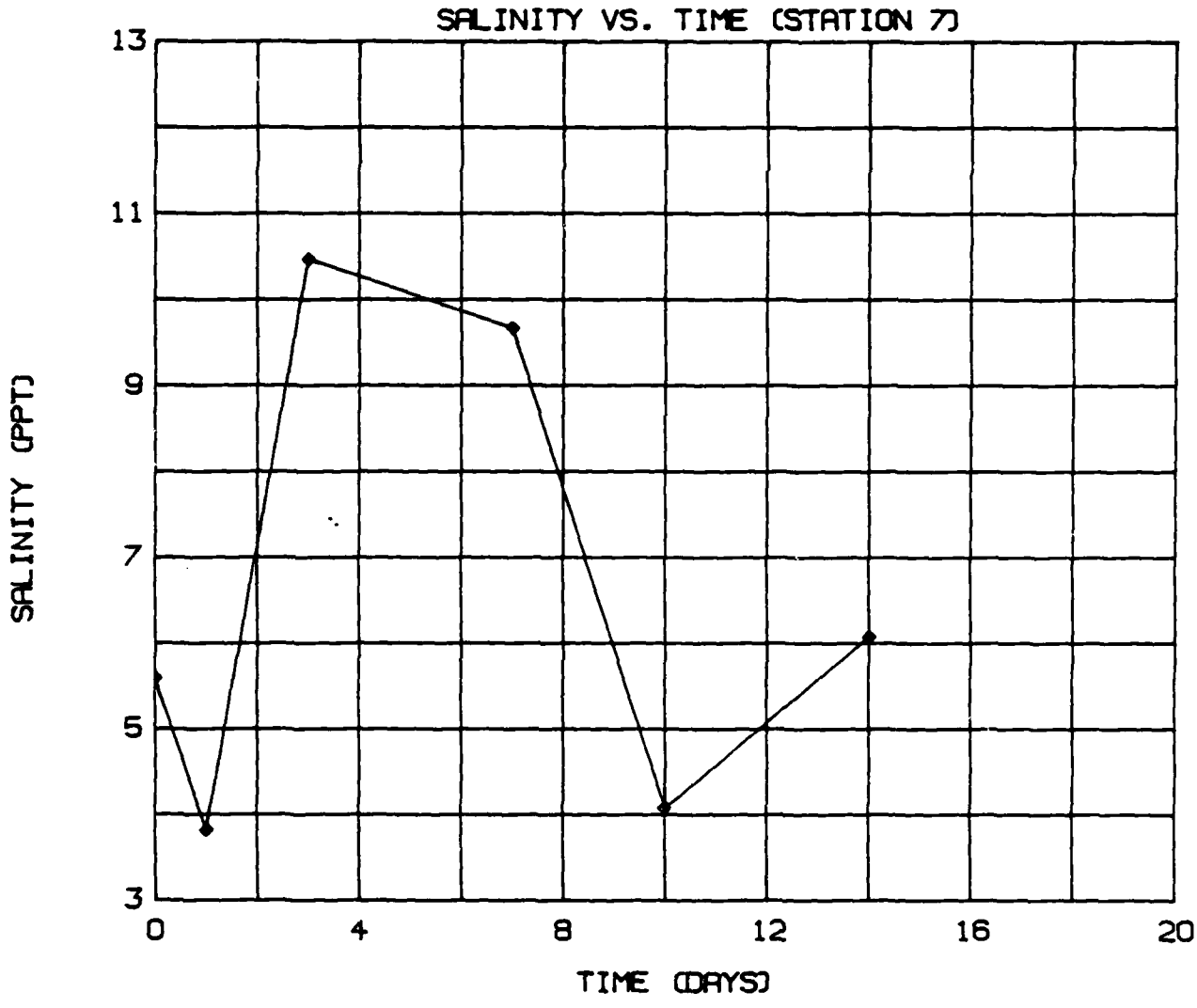
- FIGURE (17) -


SALINITY VS. TIME (STATION 5)
13.
12.6
12.2
11.8
11.4
11
SALINITY (PPT)
0
4
8
12
16
20
TIME (DAYS)

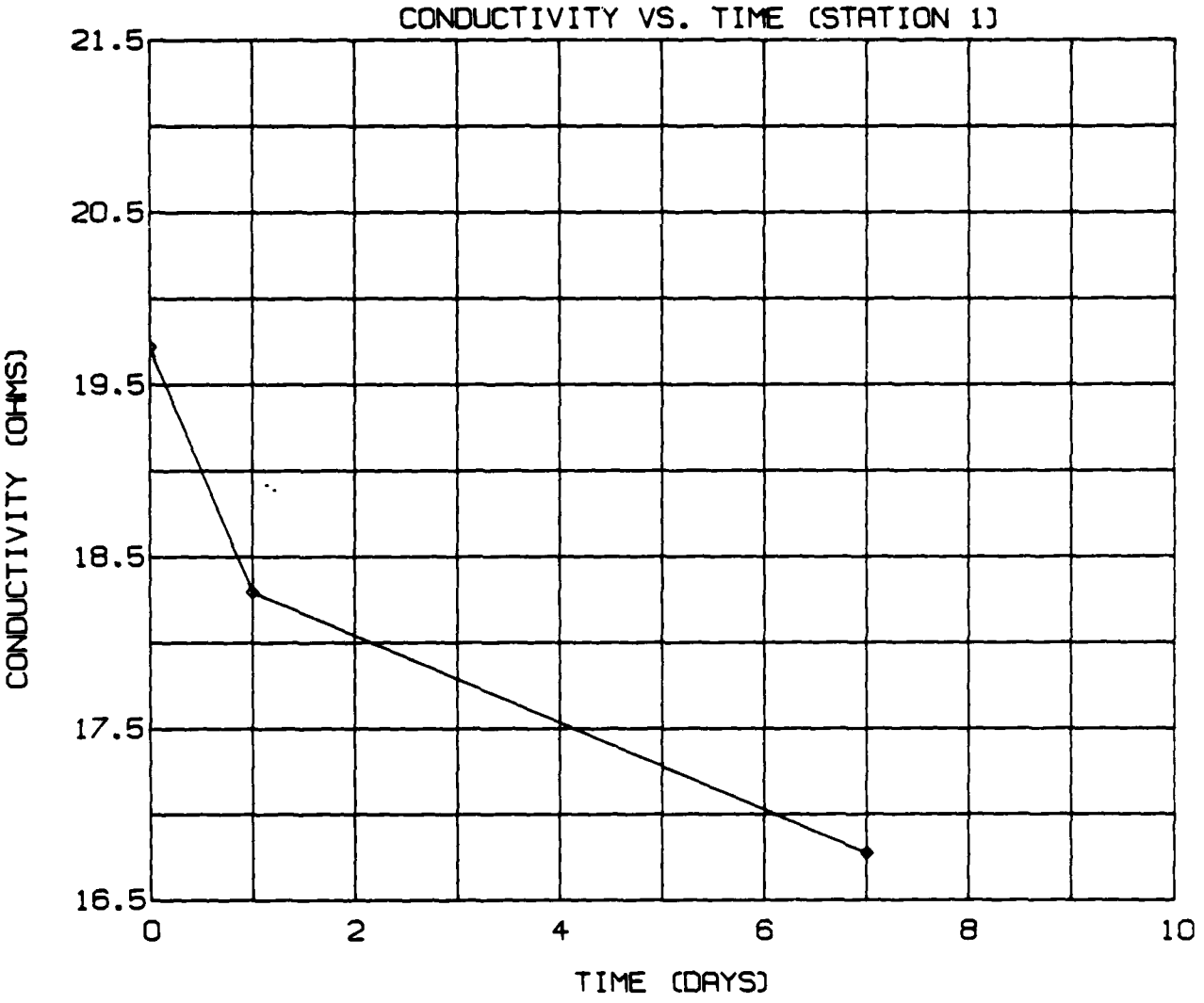
- FIGURE (18) -


SALINITY VS. TIME (STATION 6)
11.5
10.5
9.5
8.5
7.5
6.5
SALINITY (PPT)
0
4
8
12
16
20
TIME (DAYS)

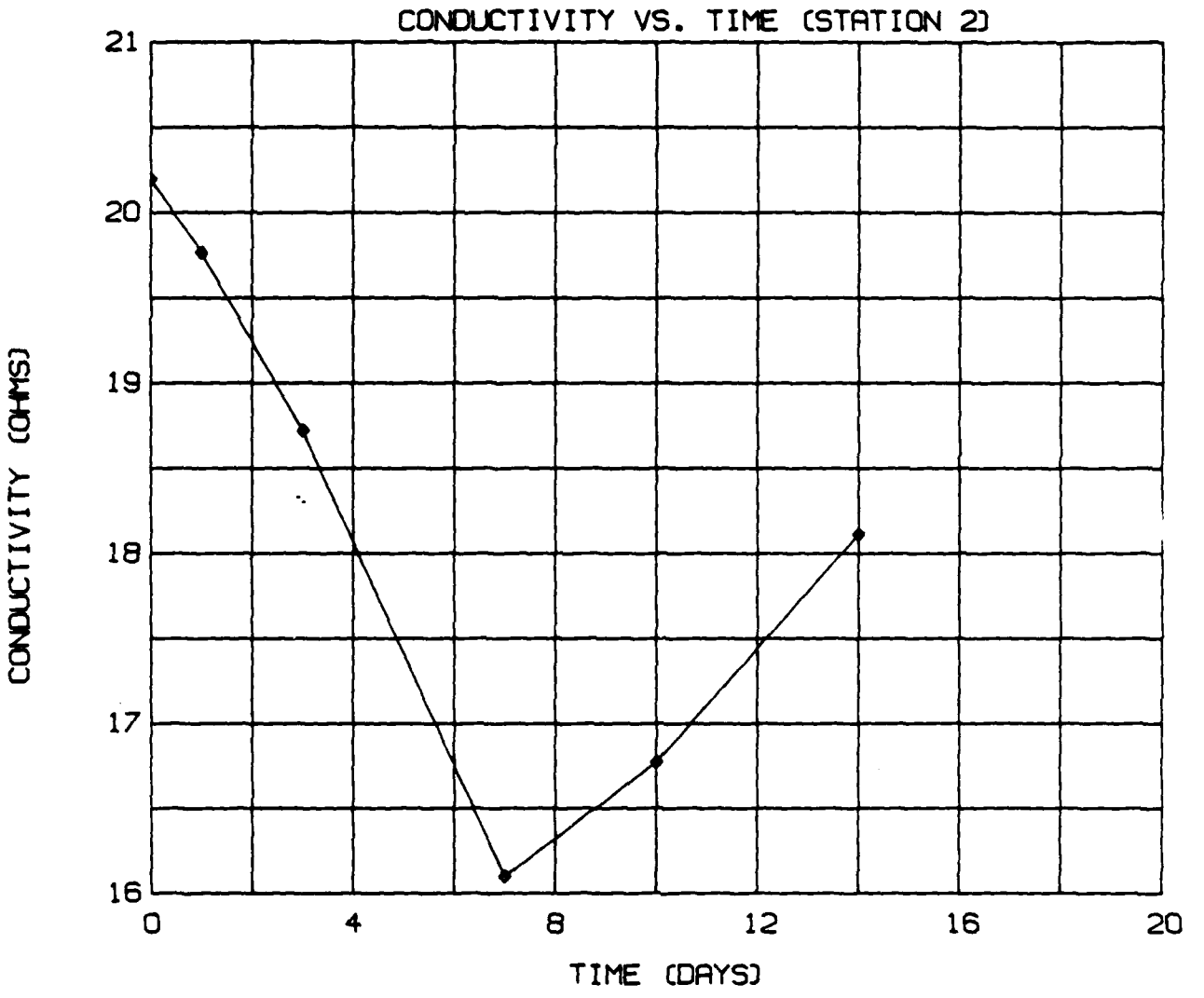
- FIGURE (19) -


SALINITY VS. TIME (STATION 7)
SALINITY (PPT)
13
11
9
7
5
3
0
4
8
12
16
20
TIME (DAYS)

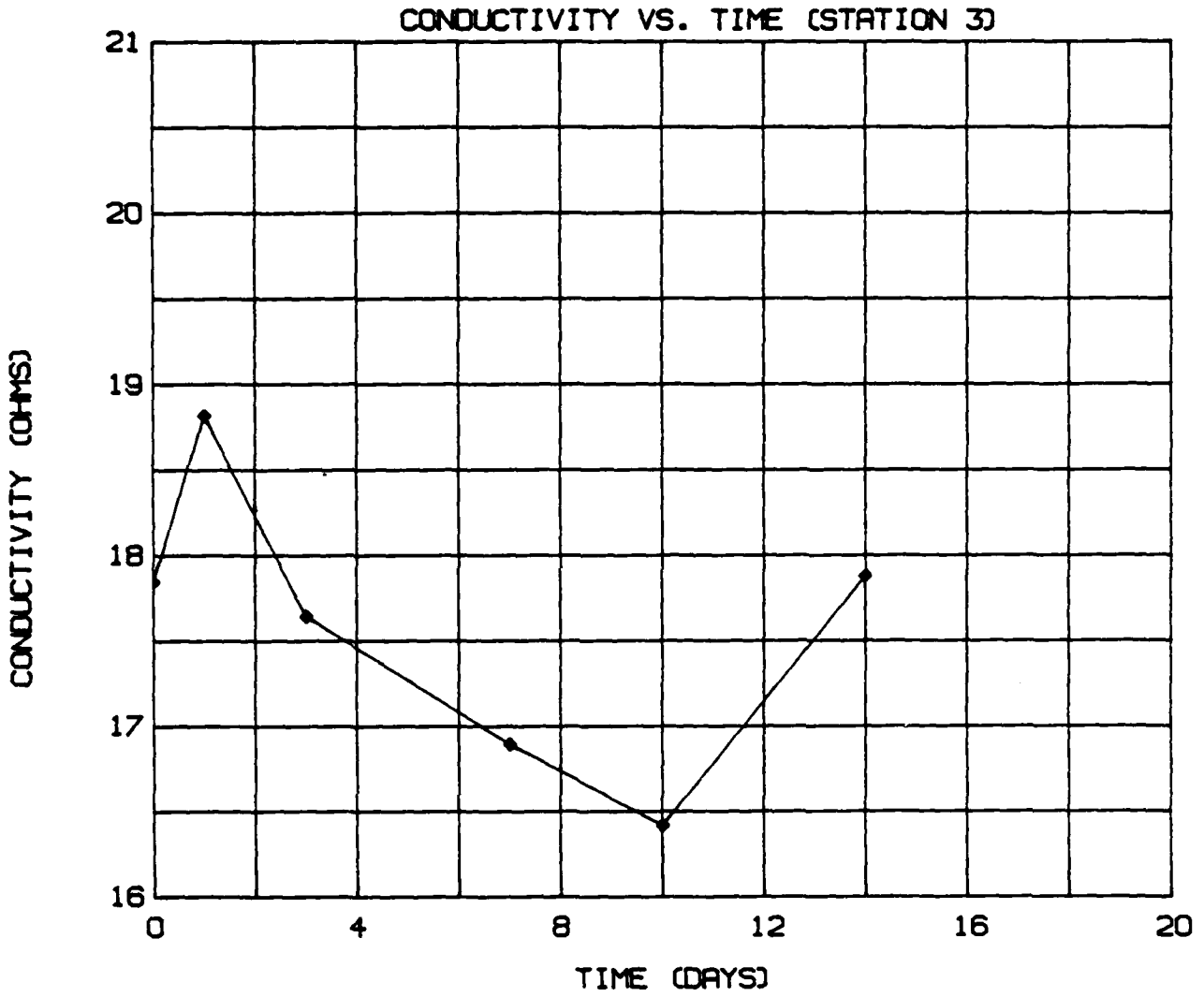
- FIGURE (20)


CONDUCTIVITY VS. TIME (STATION 1)
21.5
20.5
19.5
18.5
17.5
16.5
CONDUCTIVITY (OHMS)
0
2
4
6
8
10
TIME (DAYS)

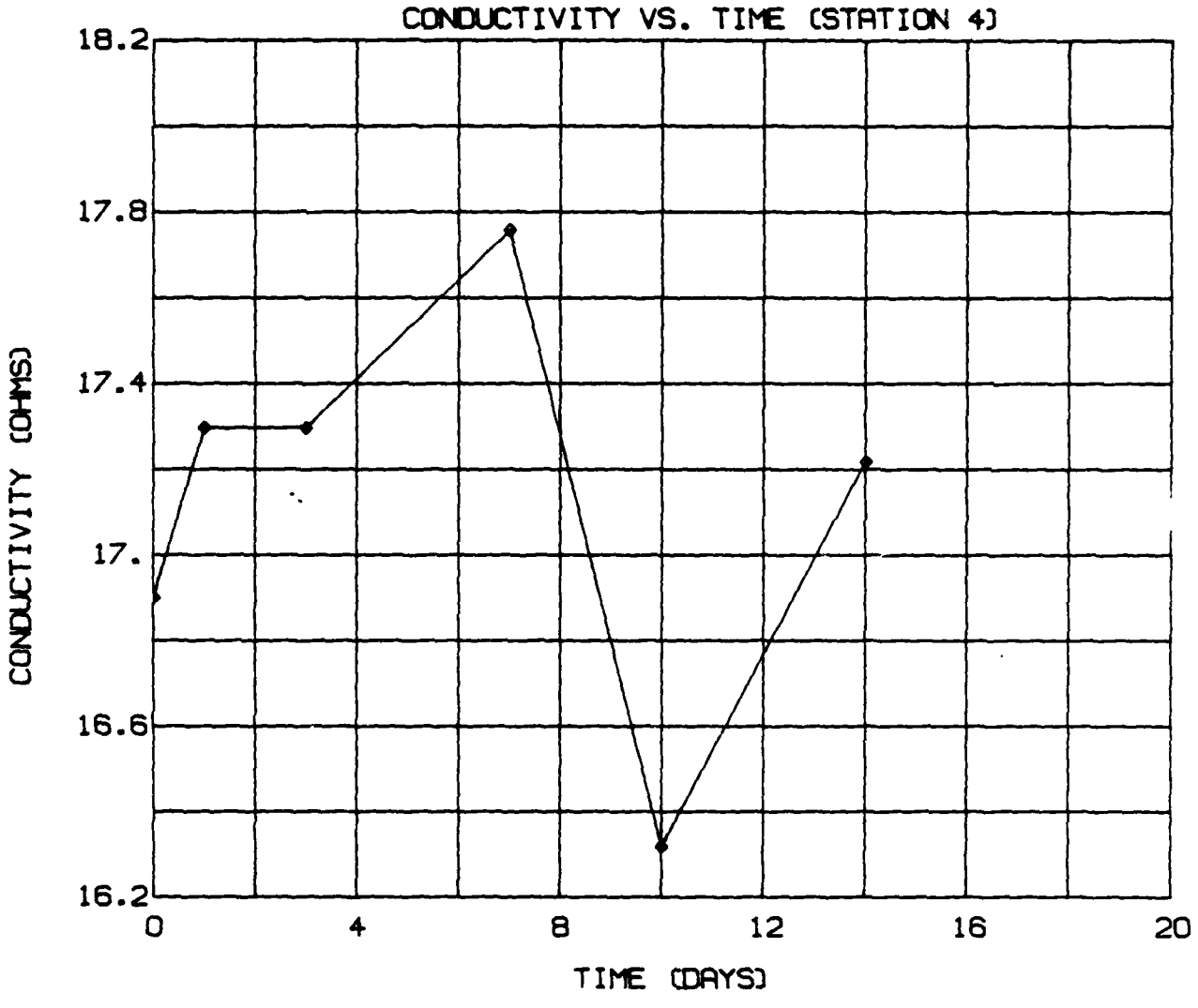
- FIGURE (21) -


CONDUCTIVITY VS. TIME (STATION 2)
CONDUCTIVITY (OHMS)
21
20
19
18
17
16
0
4
8
12
16
20
TIME (DAYS)

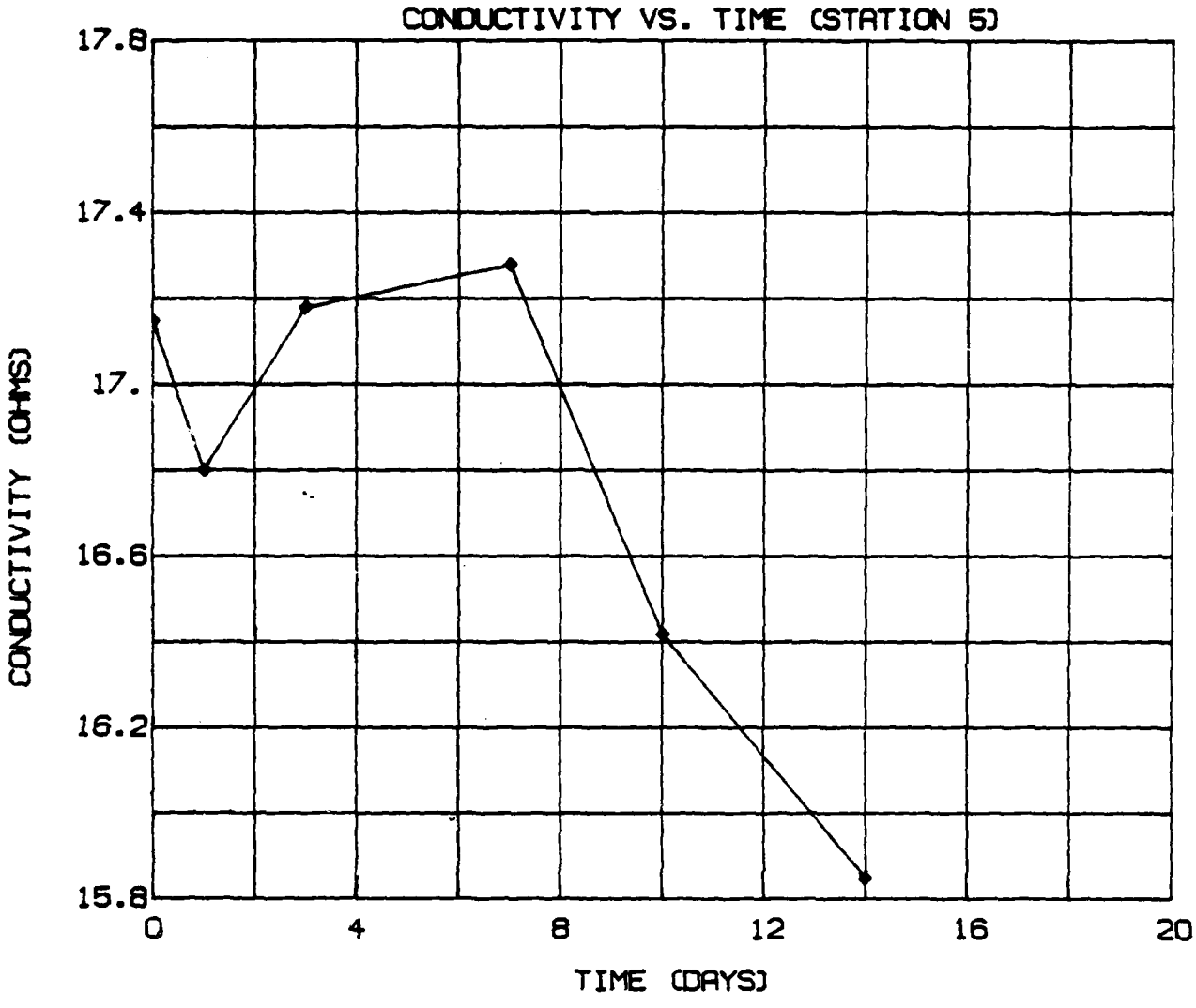
- FIGURE (22) -


CONDUCTIVITY VS. TIME (STATION 3)
CONDUCTIVITY (OHMS)
21
20
19
18
17
16
0
4
8
12
16
20
TIME (DAYS)

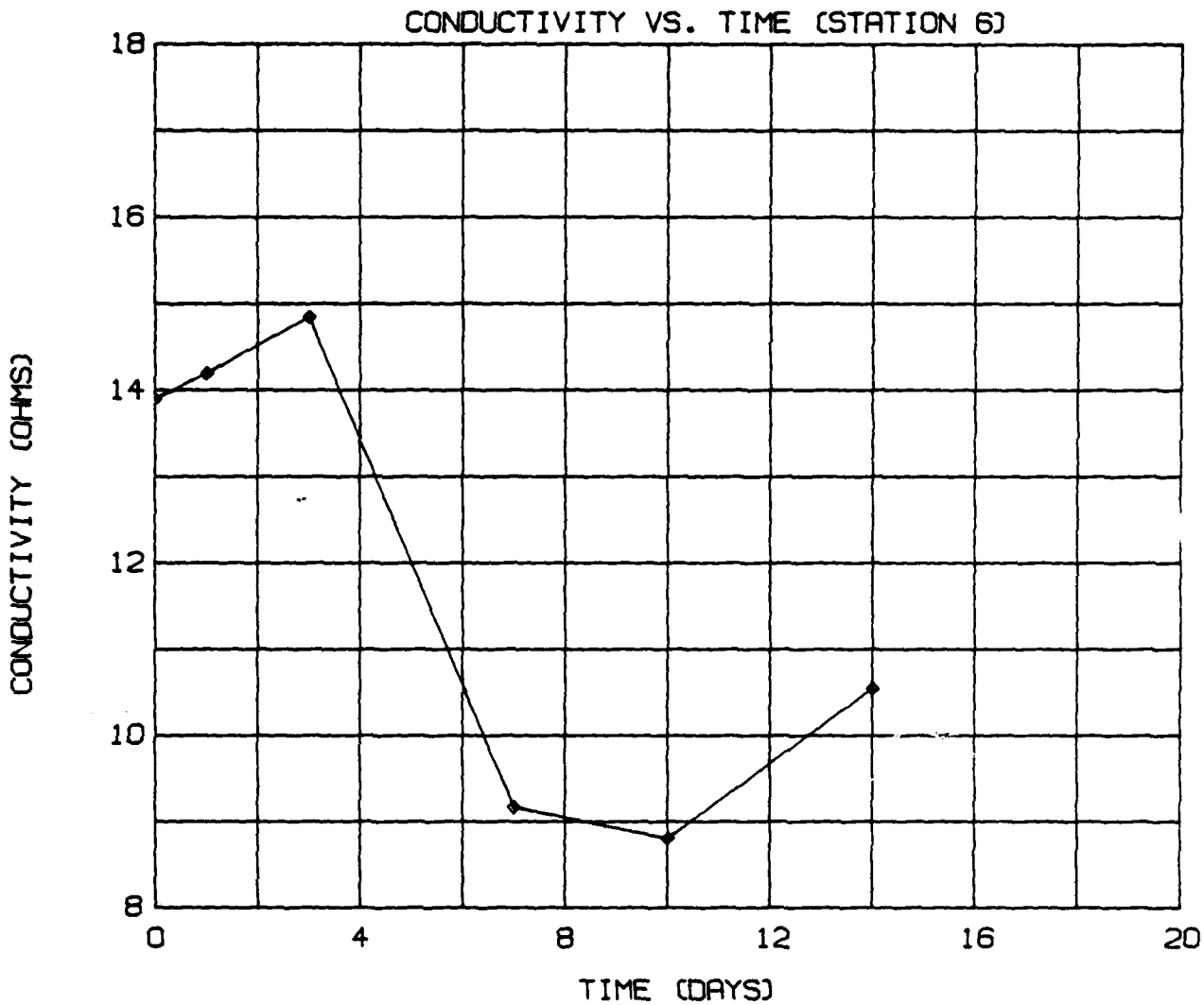
- FIGURE (23) -


CONDUCTIVITY VS. TIME (STATION 4)
18.2
17.8
17.4
17.
16.6
16.2
CONDUCTIVITY (OHMS)
0
4
8
12
16
20
TIME (DAYS)

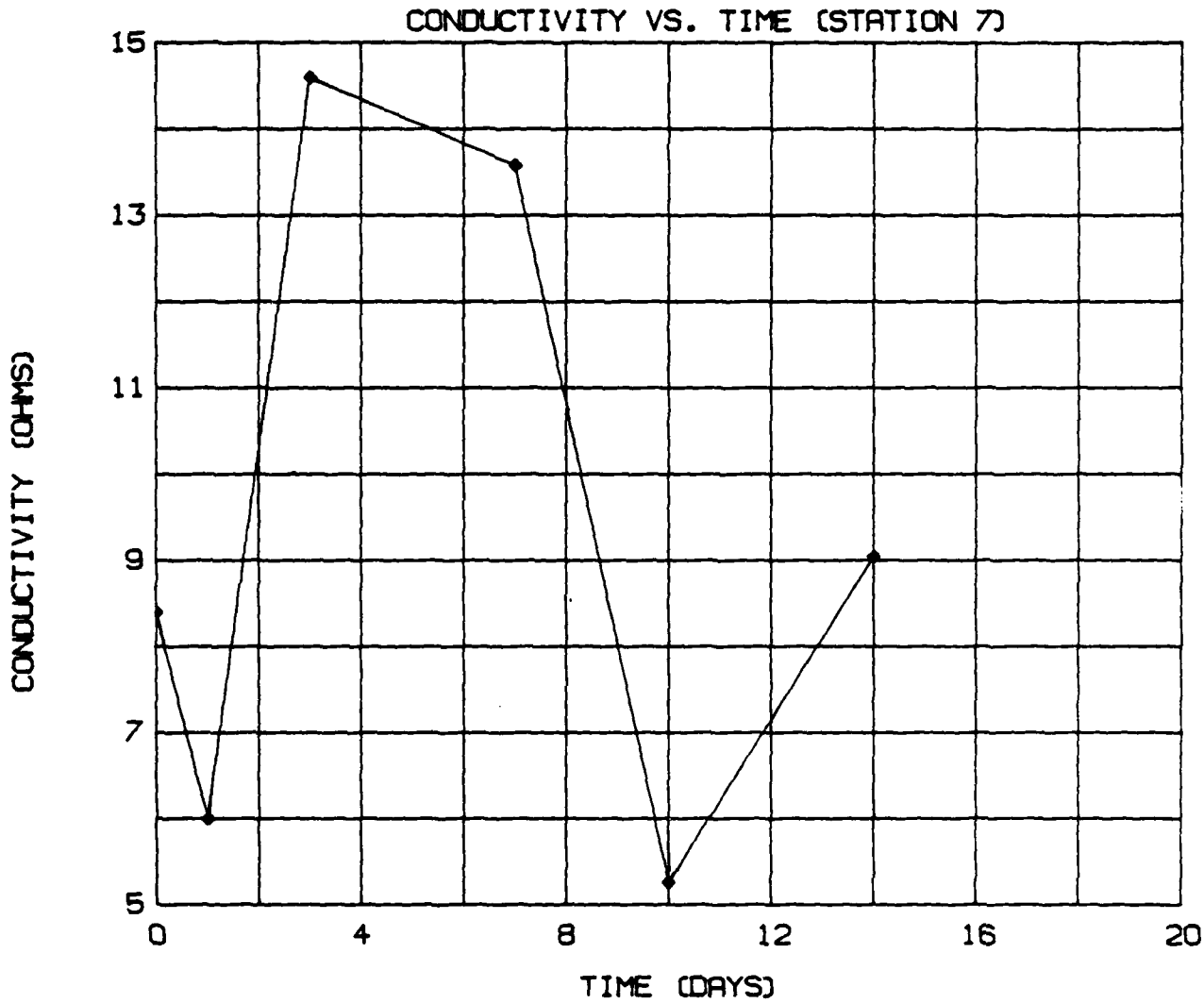
- FIGURE (24) -


CONDUCTIVITY VS. TIME (STATION 5)
17.8
17.4
17.
16.6
16.2
15.8
CONDUCTIVITY (OHMS)
0
4
8
12
16
20
TIME (DAYS)

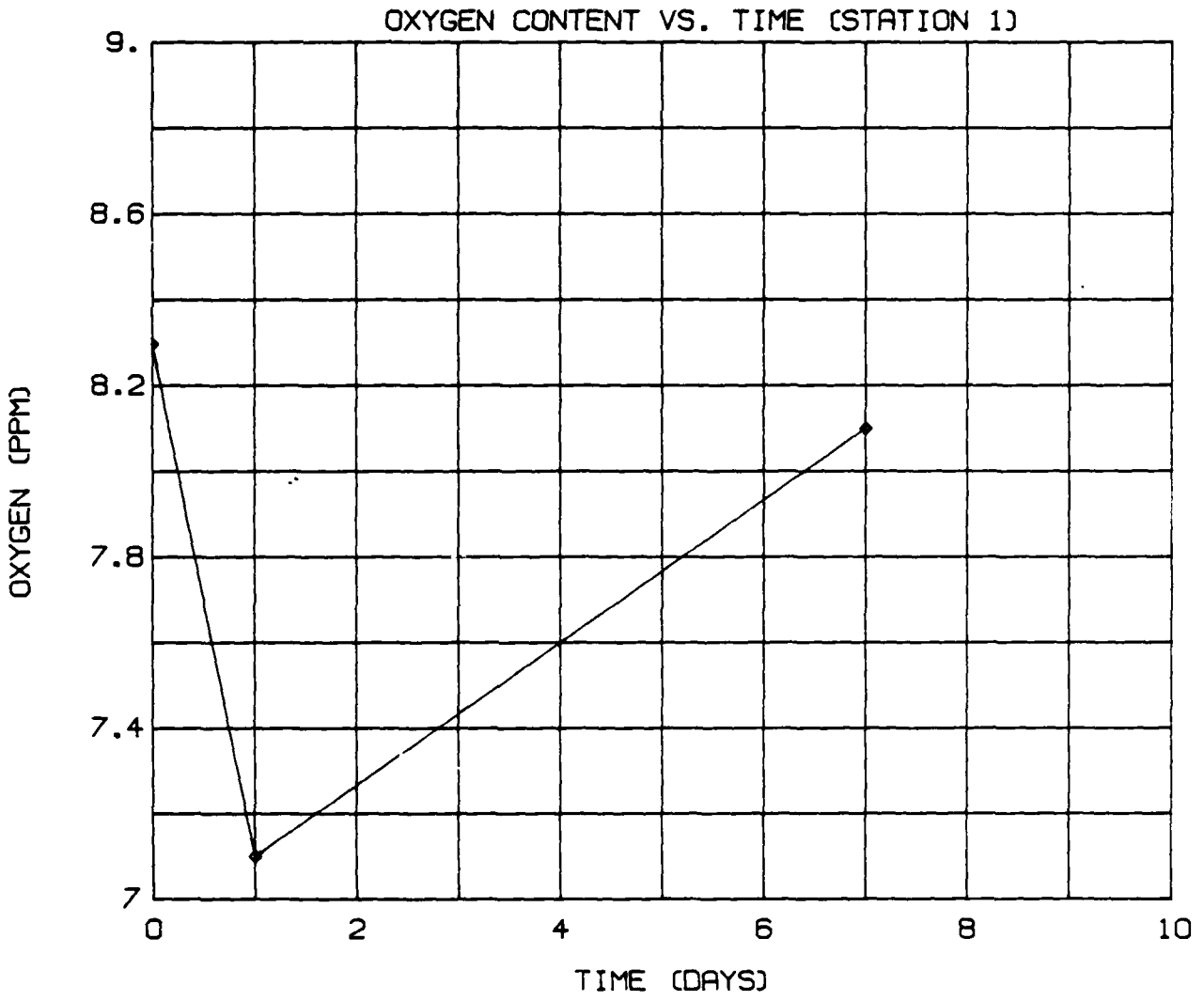
- FIGURE (25) -


CONDUCTIVITY VS. TIME (STATION 6)
CONDUCTIVITY (OHMS)
18
16
14
12
10
8
0
4
8
12
16
20
TIME (DAYS)

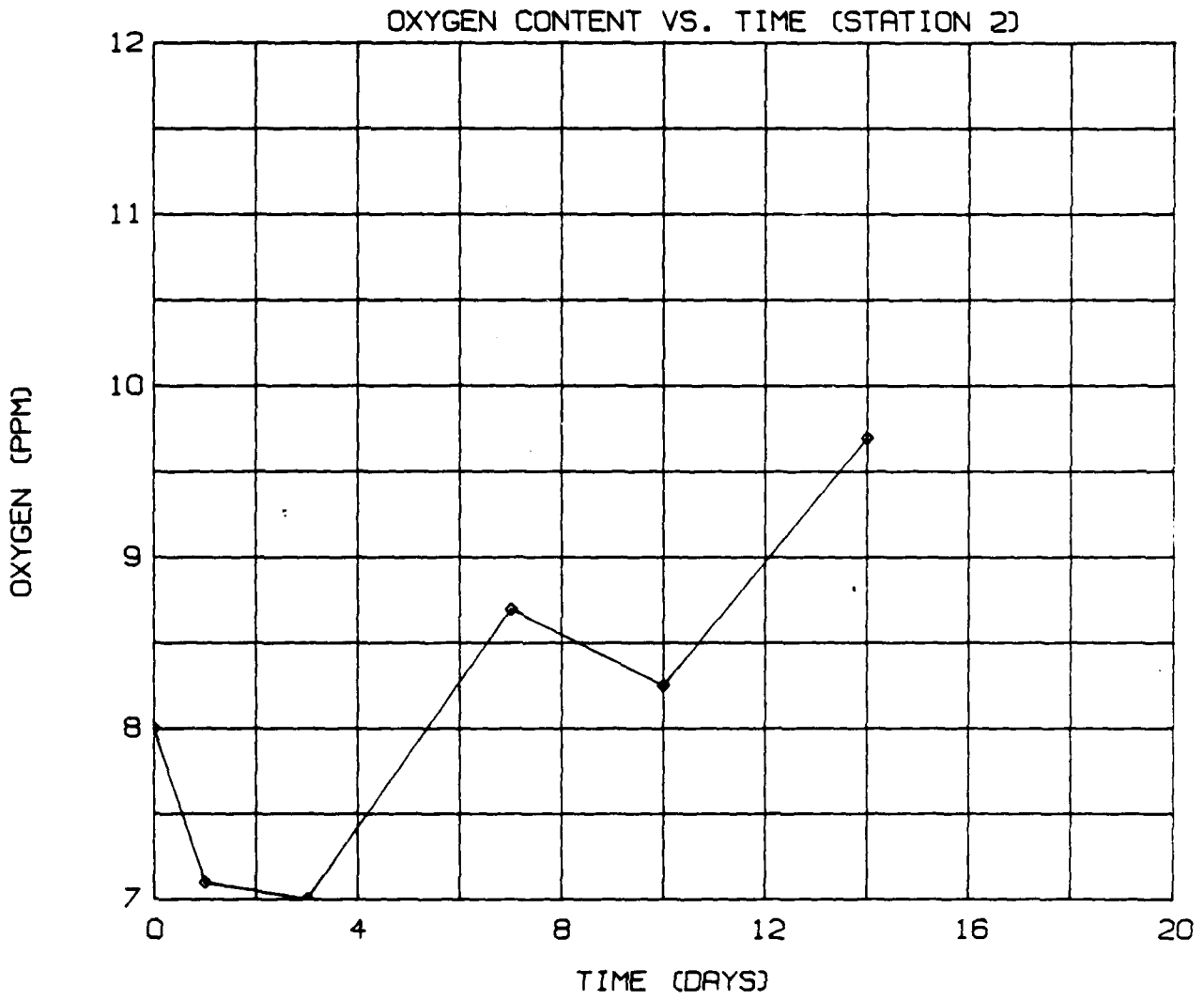
- FIGURE (26) -


CONDUCTIVITY VS. TIME (STATION 7)
CONDUCTIVITY (OHMS)
15
13
11
9
7
5
0
4
8
12
16
20
TIME (DAYS)

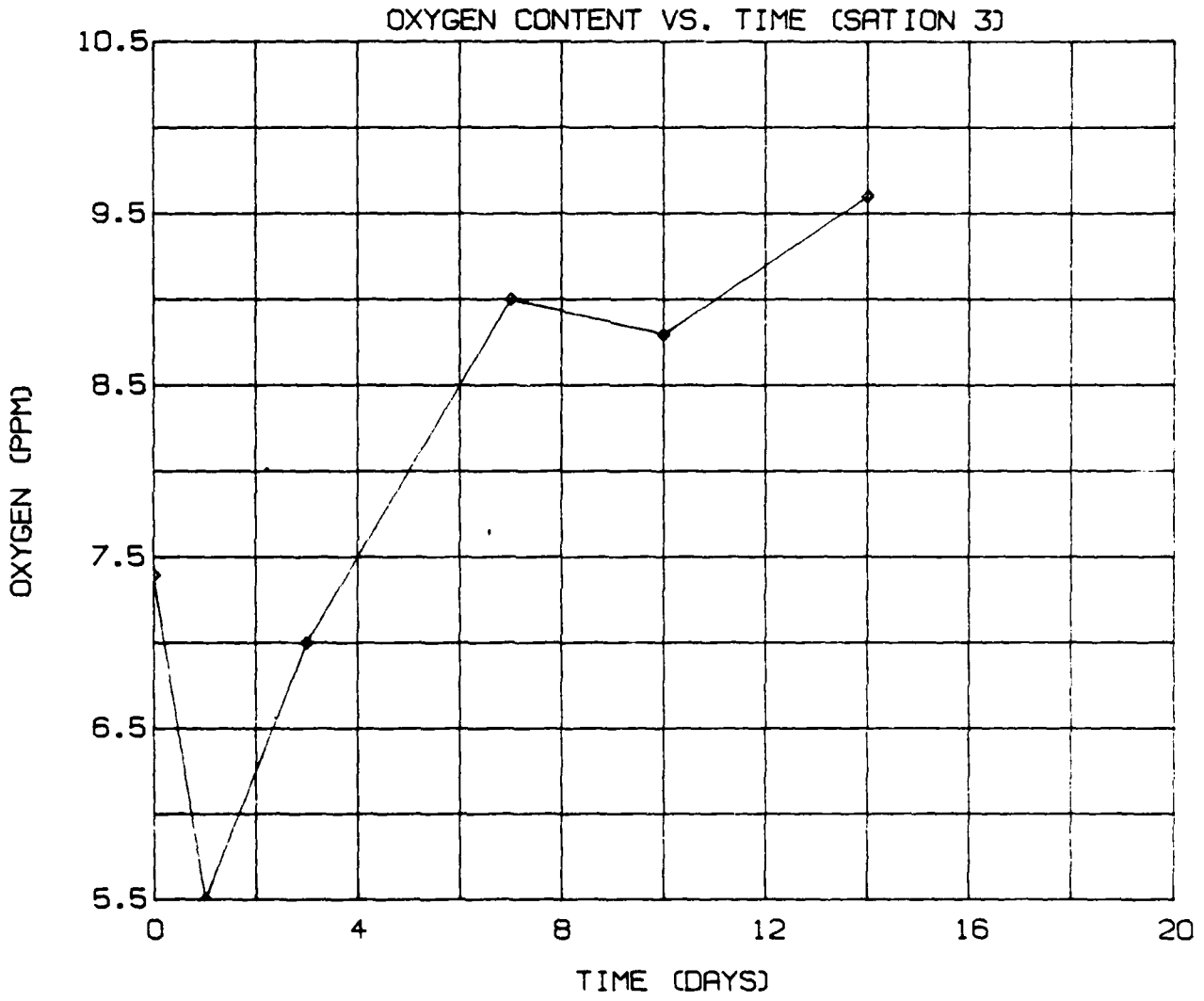
- FIGURE (27) -


OXYGEN CONTENT VS. TIME (STATION 1)
9.
8.6
8.2
7.8
7.4
7
OXYGEN (PPM)
0
2
4
6
8
10
TIME (DAYS)

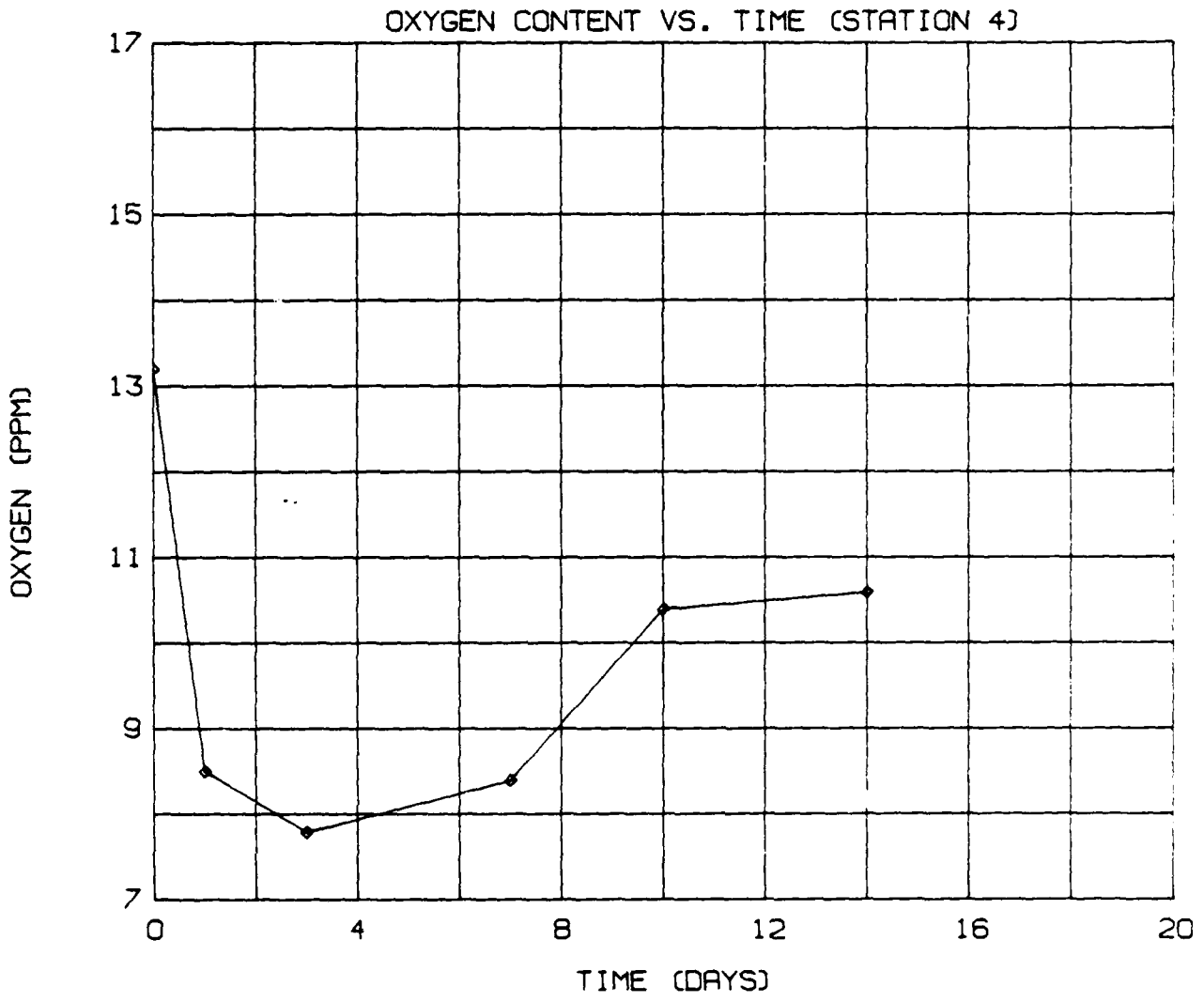
- FIGURE (28) -


OXYGEN CONTENT VS. TIME (STATION 2)
12
11
10
9
8
7
OXYGEN (PPM)
0
4
8
12
16
20
TIME (DAYS)

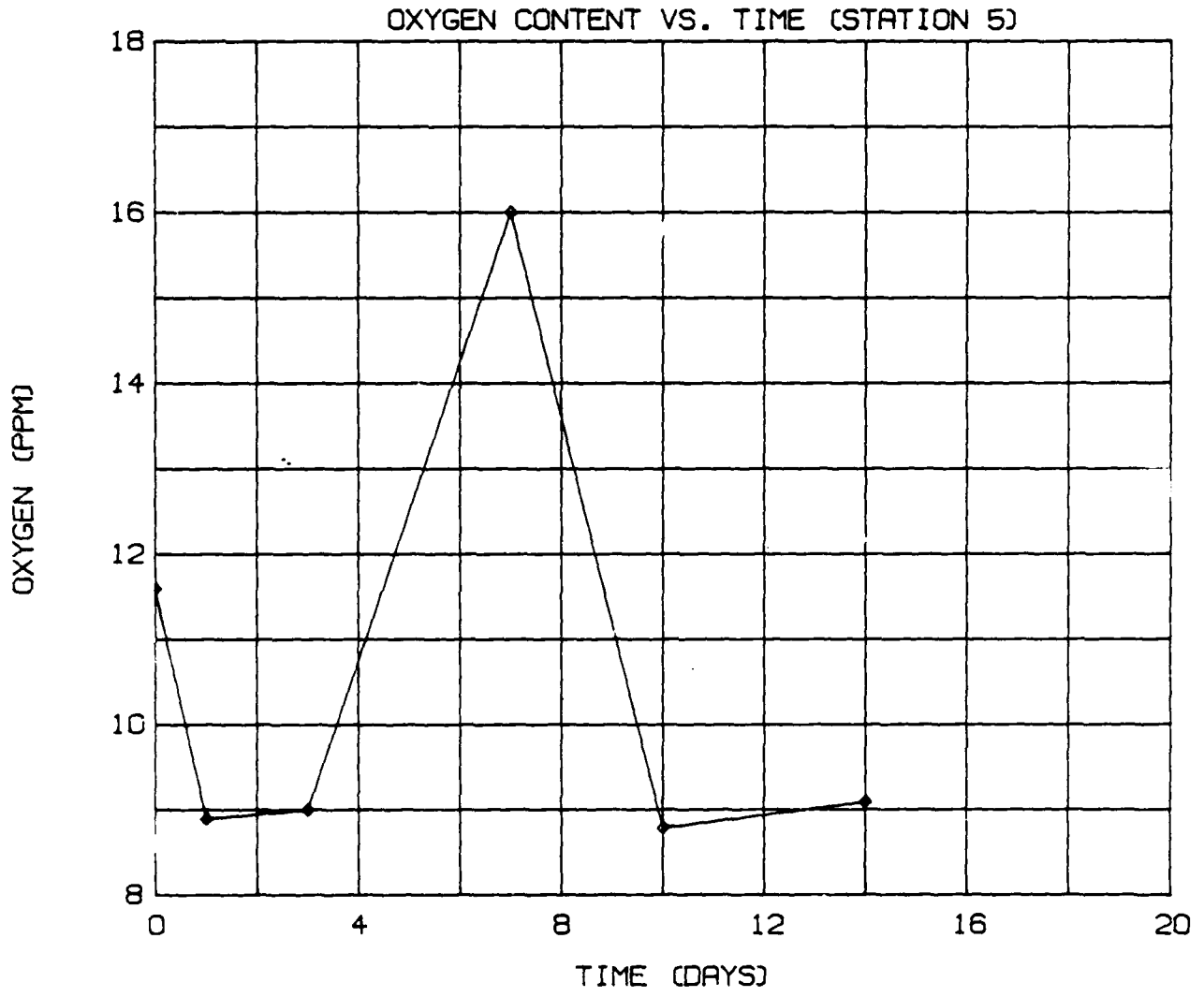
- FIGURE (29) -


OXYGEN CONTENT VS. TIME (SATION 3)
10.5
9.5
8.5
7.5
6.5
5.5
OXYGEN (PPM)
0
4
8
12
16
20
TIME (DAYS)

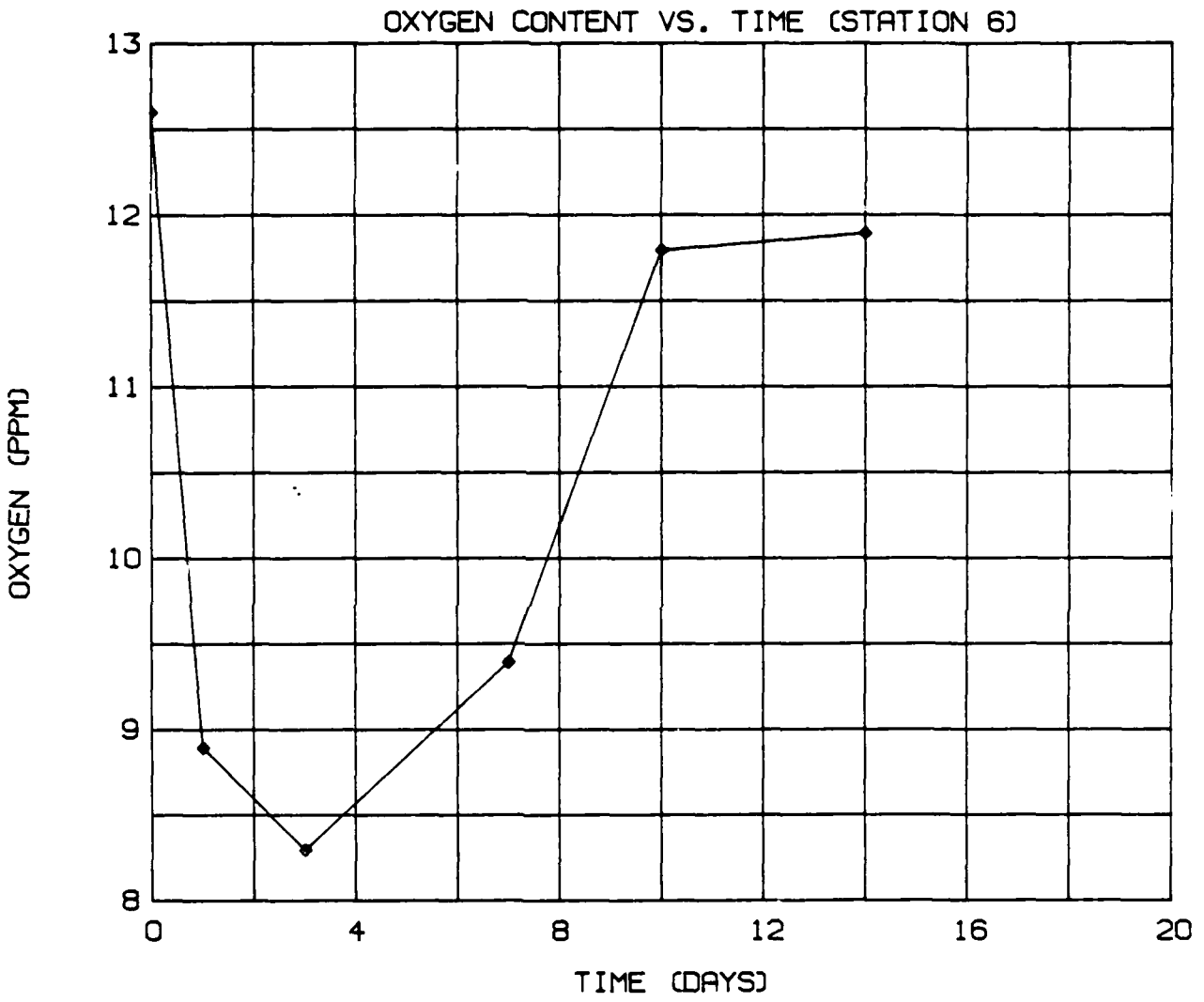
- FIGURE (30) -


OXYGEN CONTENT VS. TIME (STATION 4)
OXYGEN (PPM)
17
15
13
11
9
7
0
4
8
12
16
20
TIME (DAYS)

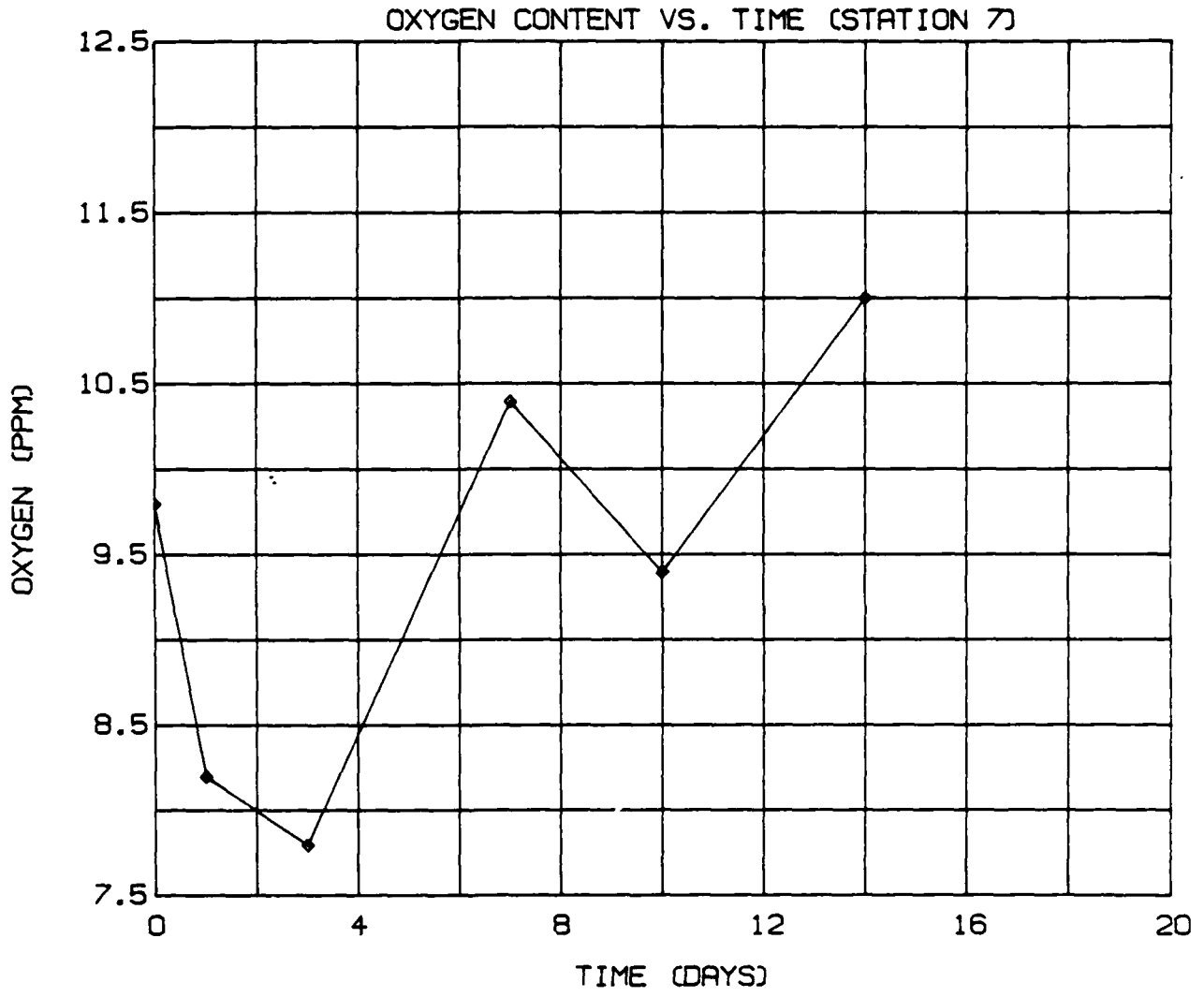
- FIGURE (31) -


OXYGEN CONTENT VS. TIME (STATION 5)
OXYGEN (PPM)
18
16
14
12
10
8
0
4
8
12
16
20
TIME (DAYS)

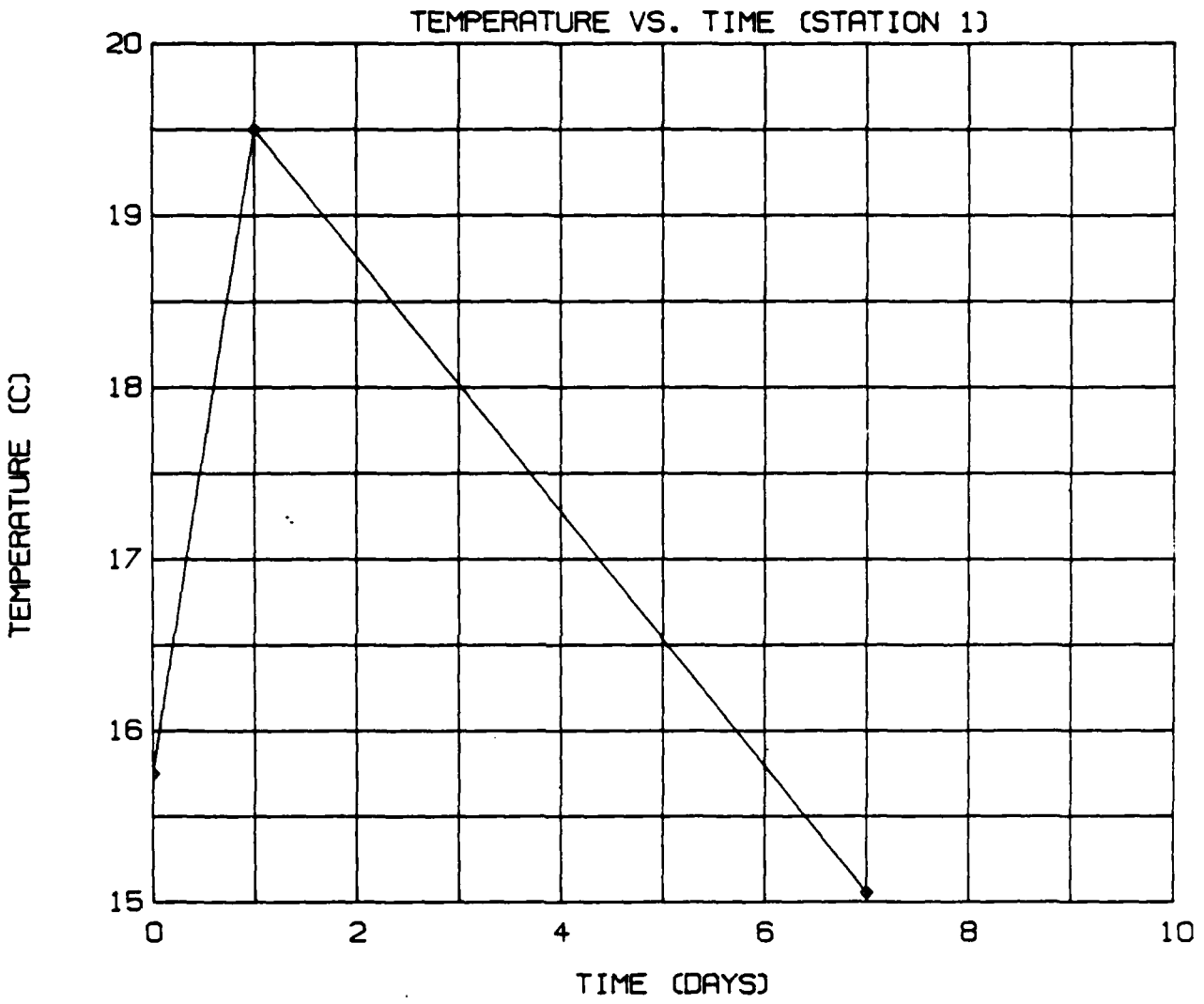
- FIGURE (32) -


OXYGEN CONTENT VS. TIME (STATION 6)
OXYGEN (PPM)
13
12
11
10
9
8
0
4
8
12
16
20
TIME (DAYS)

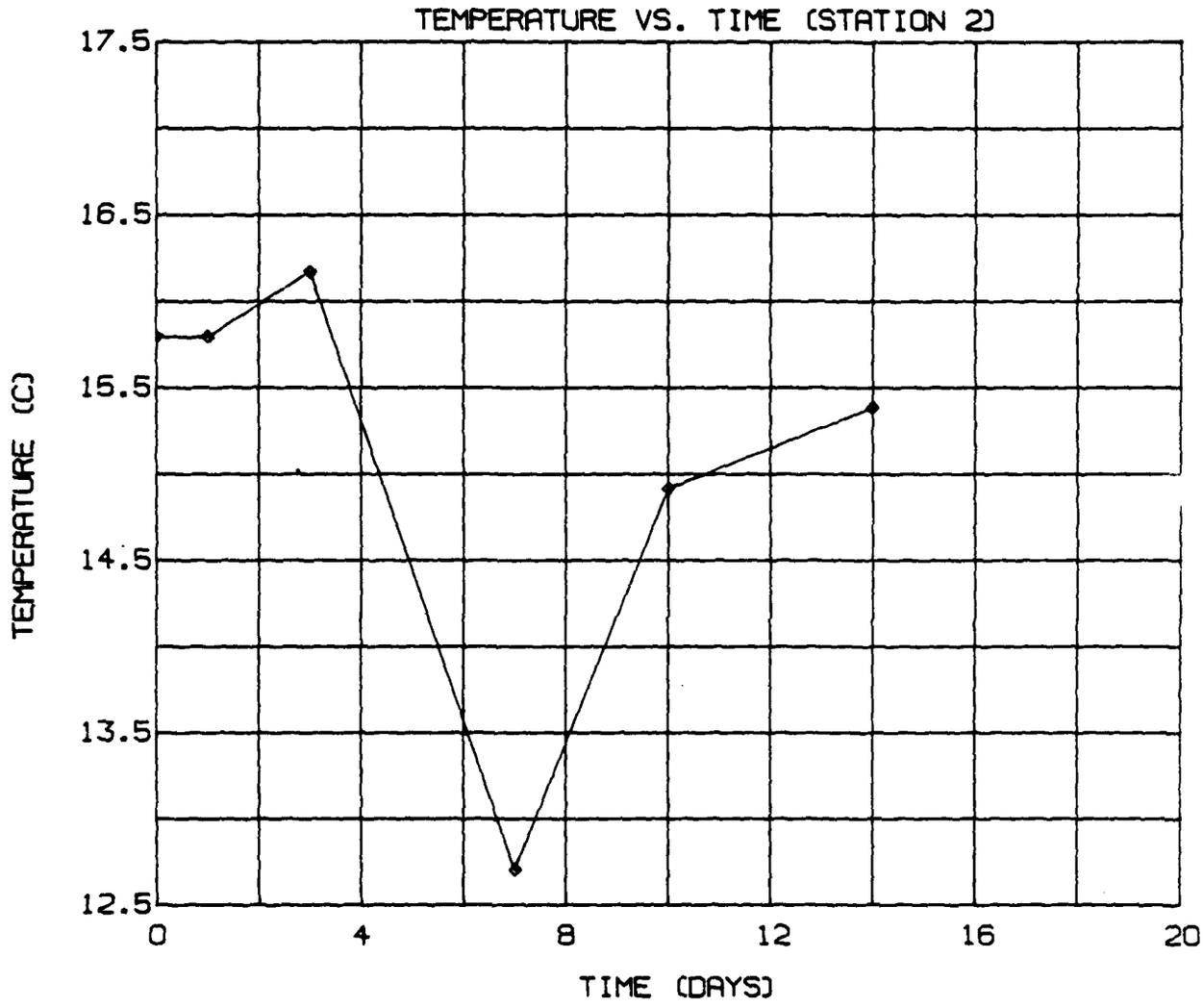
- FIGURE (33) -


OXYGEN CONTENT VS. TIME (STATION 7)
12.5
11.5
10.5
9.5
8.5
7.5
OXYGEN (PPM)
0
4
8
12
16
20
TIME (DAYS)

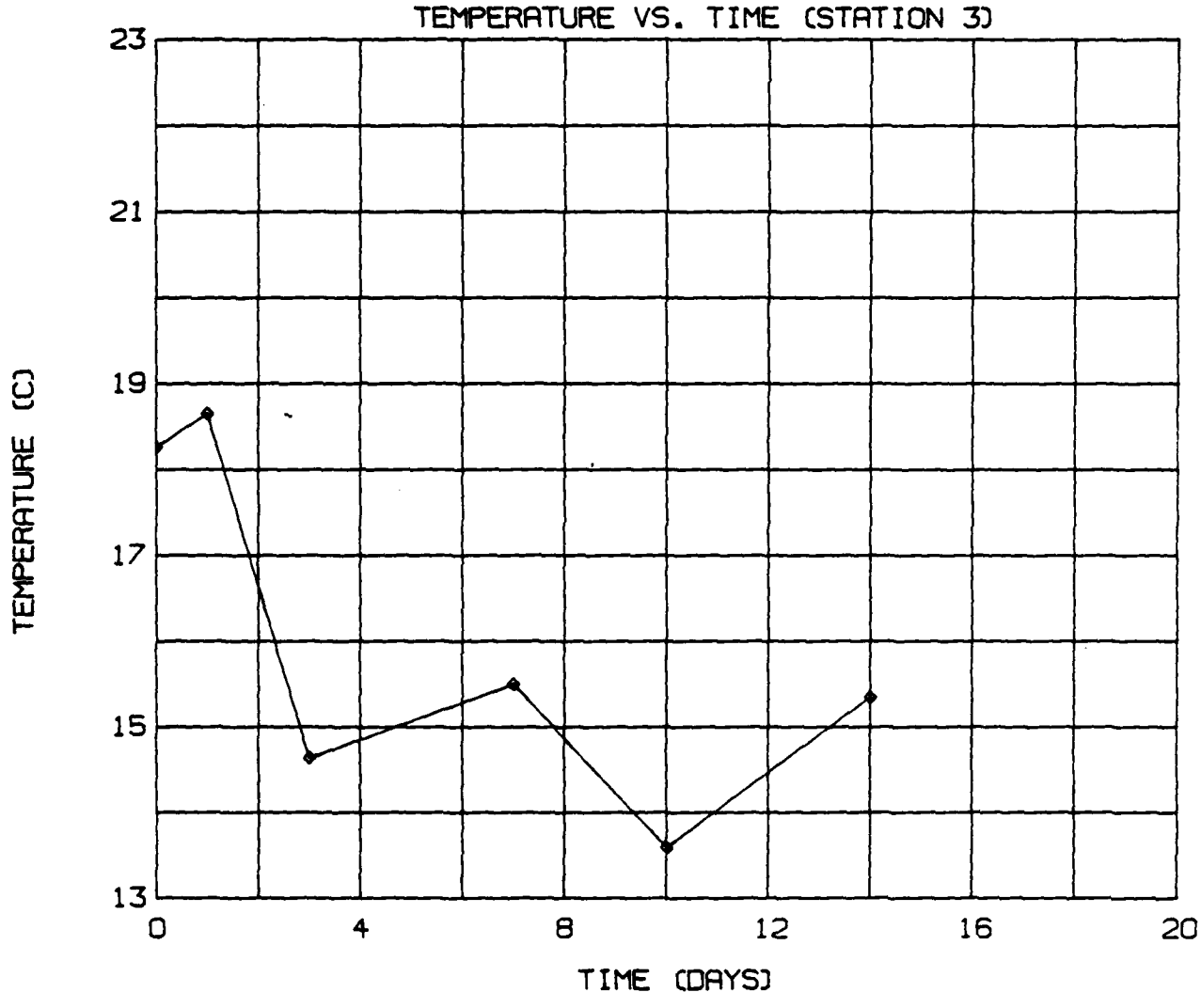
- FIGURE (34) -


TEMPERATURE VS. TIME (STATION 1)
TEMPERATURE (C)
20
19
18
17
16
15
0
2
4
6
8
10
TIME (DAYS)

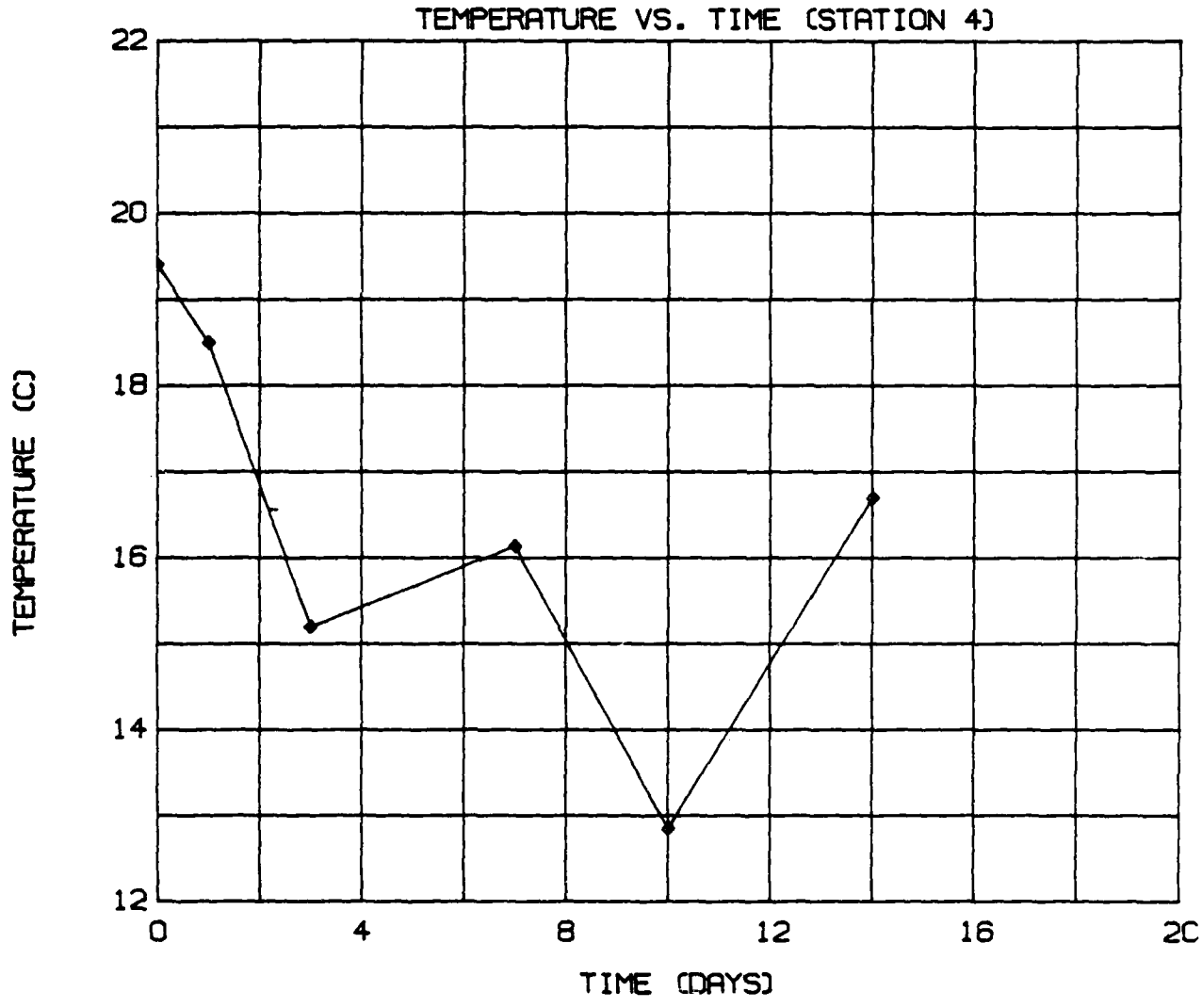
- FIGURE (35) -


TEMPERATURE VS. TIME (STATION 2)
17.5
16.5
15.5
14.5
13.5
12.5
TEMPERATURE (C)
0
4
8
12
16
20
TIME (DAYS)

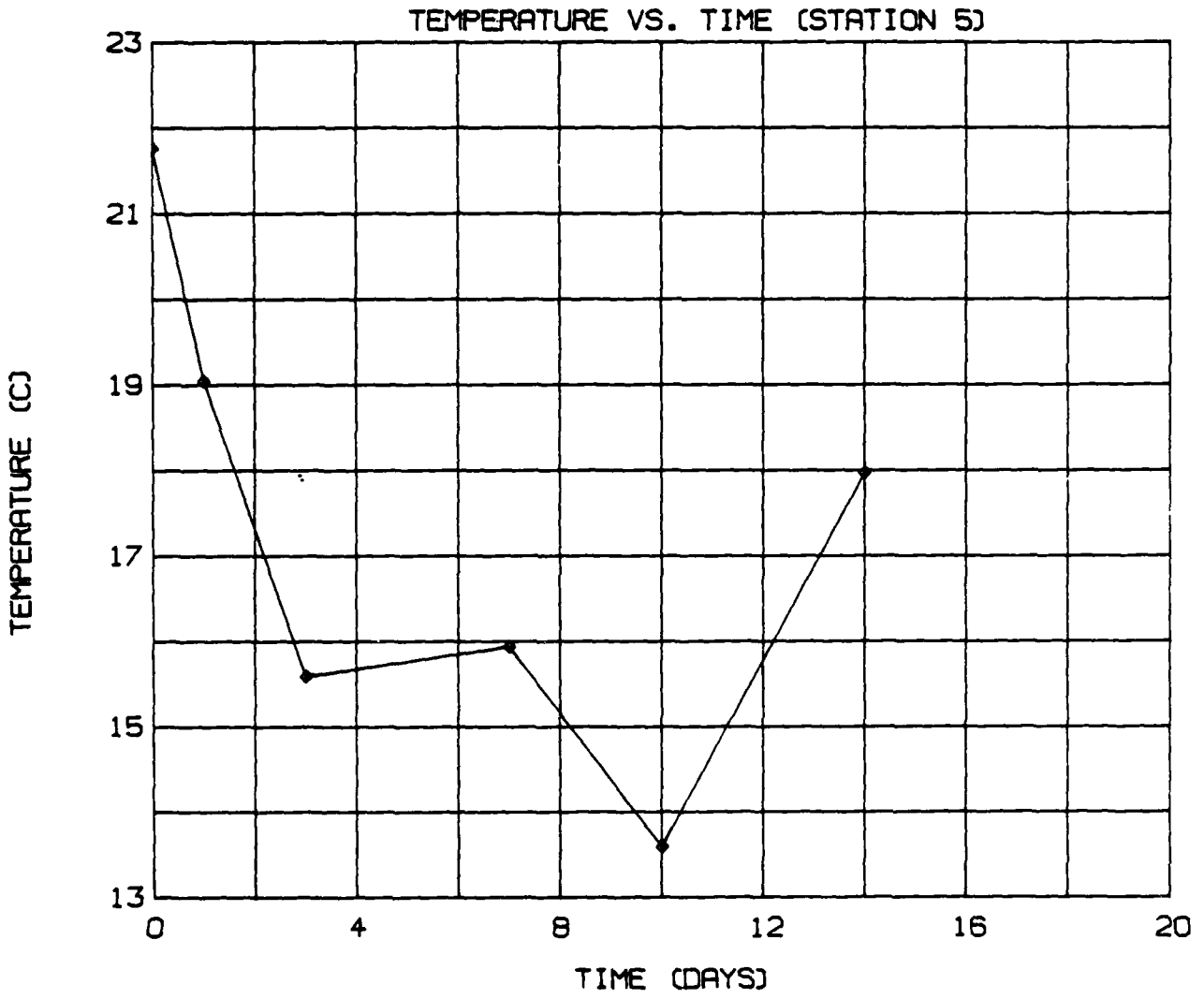
- FIGURE (36) -


TEMPERATURE VS. TIME (STATION 3)
TEMPERATURE (C)
23
21
19
17
15
13
0
4
8
12
16
20
TIME (DAYS)

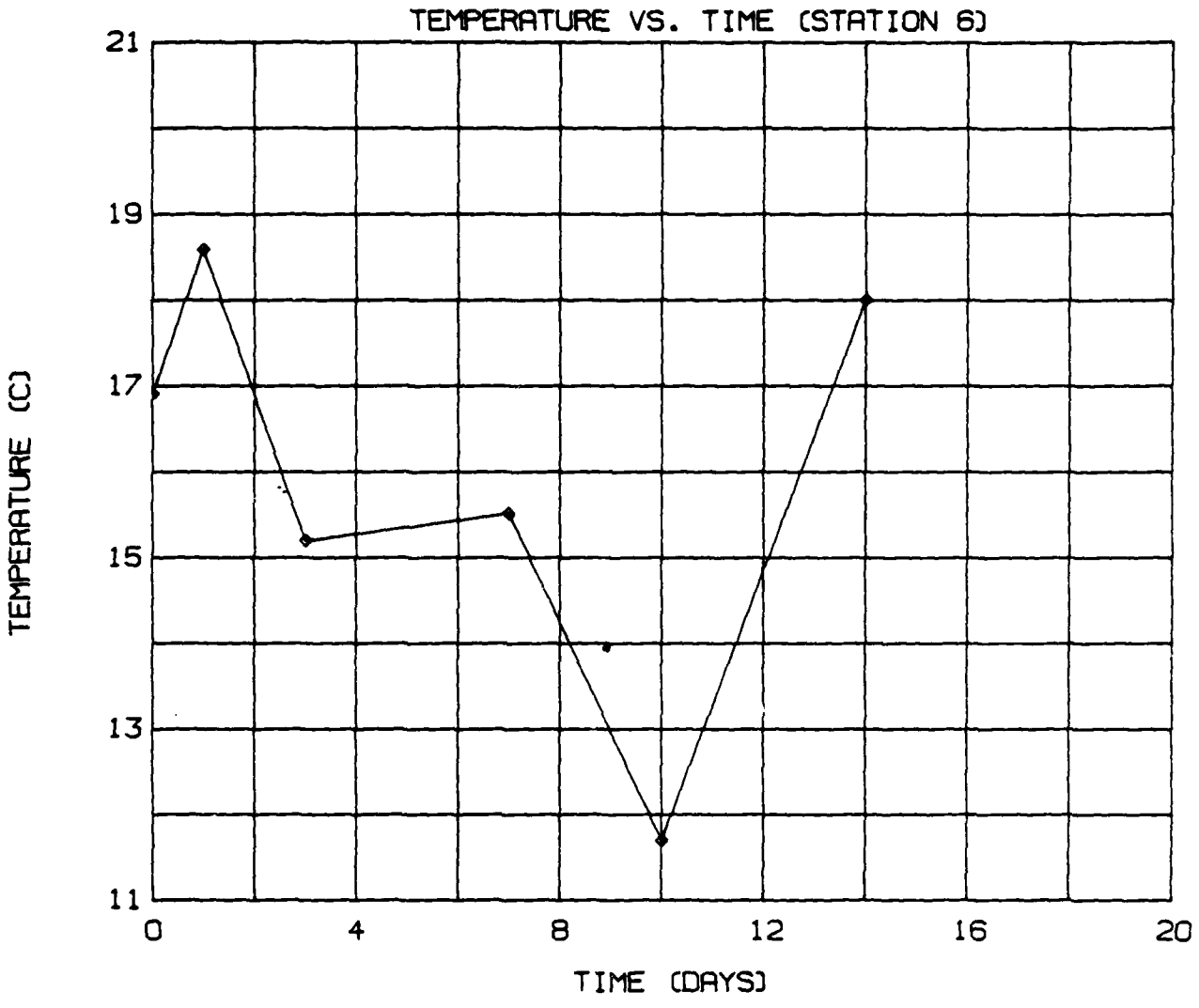
- FIGURE (37) -


TEMPERATURE VS. TIME (STATION 4)
TEMPERATURE (C)
22
20
18
16
14
12
0
4
8
12
16
20
TIME (DAYS)

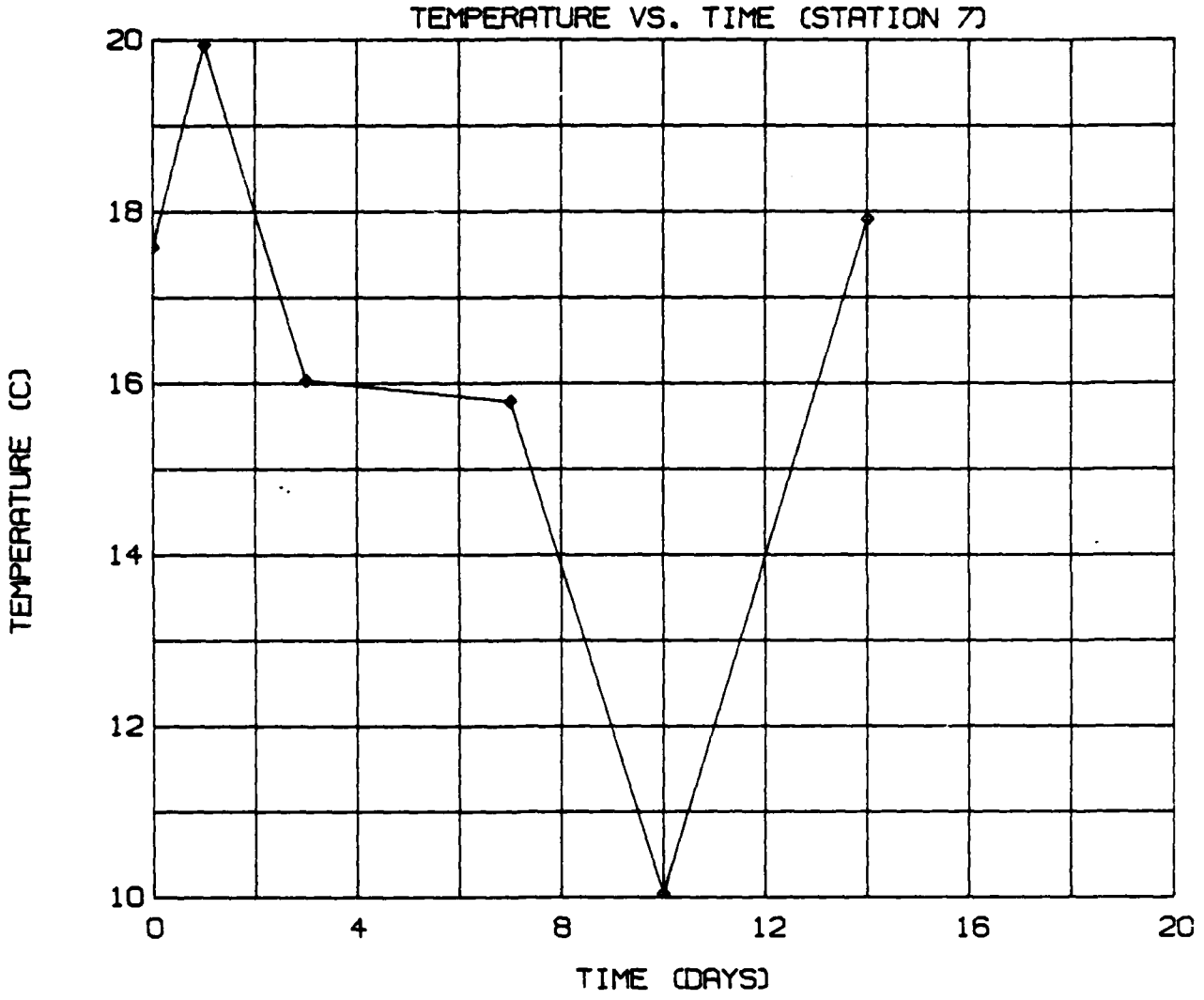
- FIGURE (38) -


TEMPERATURE VS. TIME (STATION 5)
TEMPERATURE (C)
23
21
19
17
15
13
0
4
8
12
16
20
TIME (DAYS)

- FIGURE (39) -


TEMPERATURE VS. TIME (STATION 6)
TEMPERATURE (C)
21
19
17
15
13
11
0
4
8
12
16
20
TIME (DAYS)

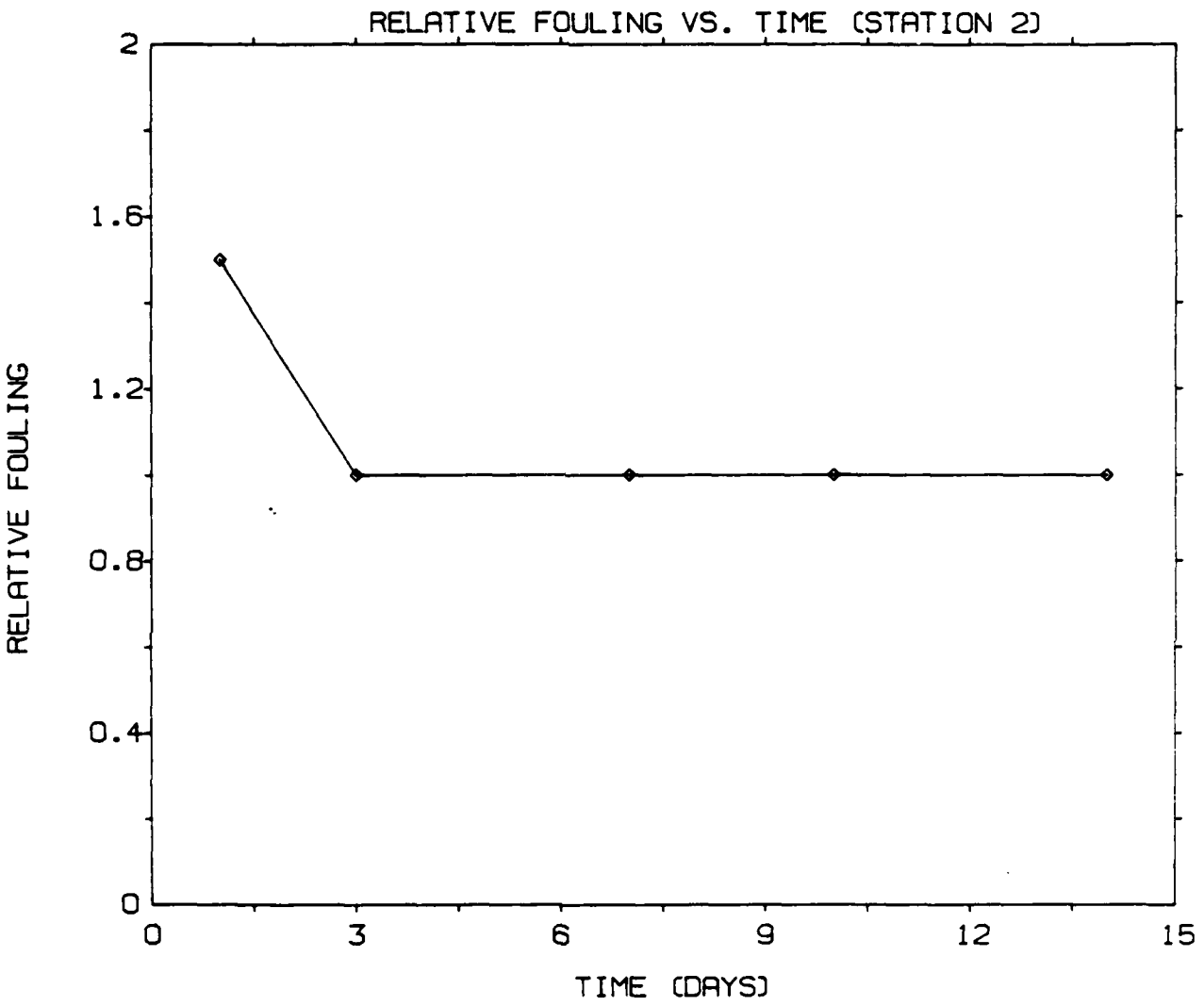
- FIGURE (40) -


TEMPERATURE VS. TIME (STATION 7)
TEMPERATURE (C)
20
18
16
14
12
10
0
4
8
12
16
20
TIME (DAYS)

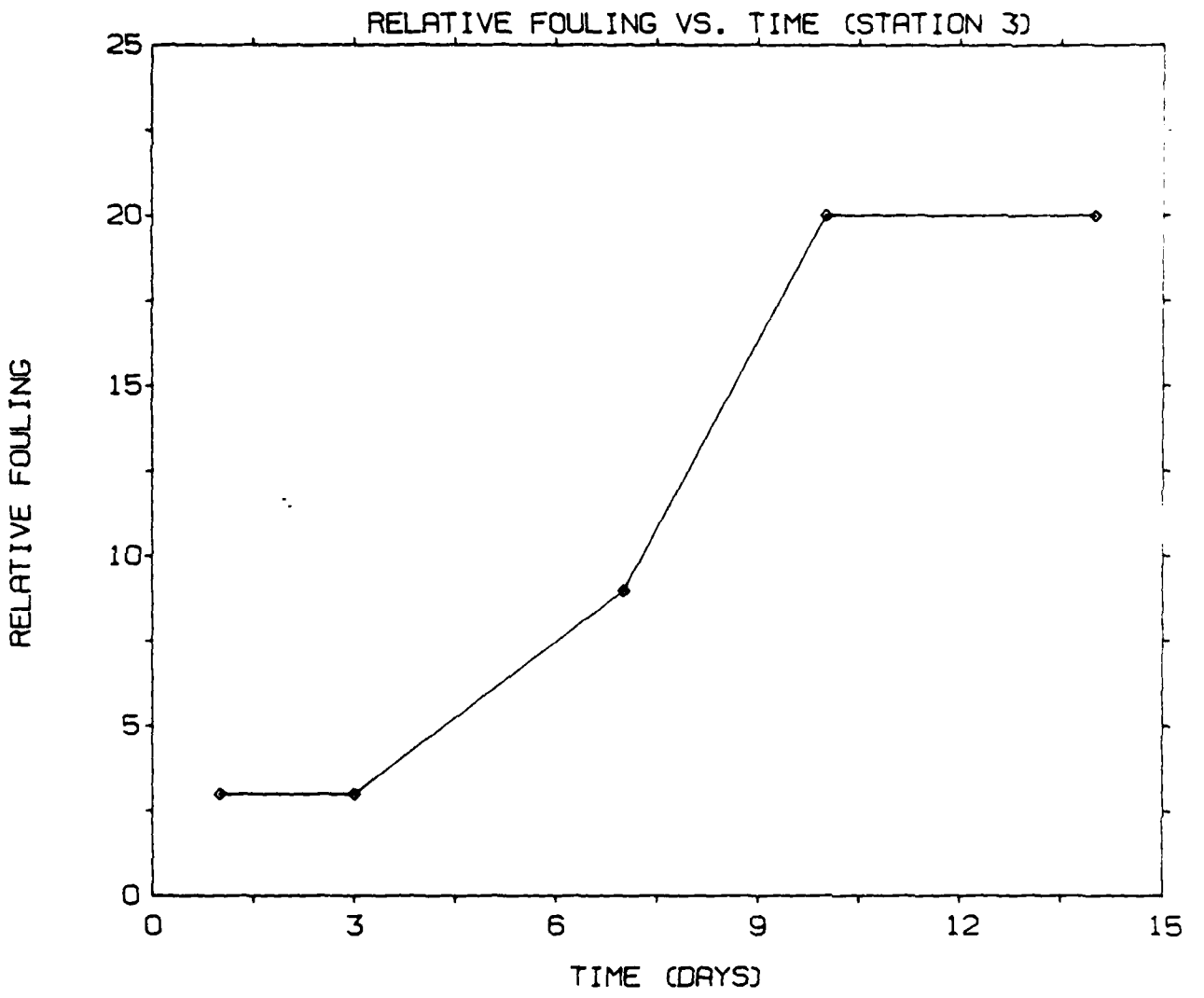
APPENDIX B - EXPERIMENT ONE:

GRAPHS OF RELATIVE FOULING VS. TIME

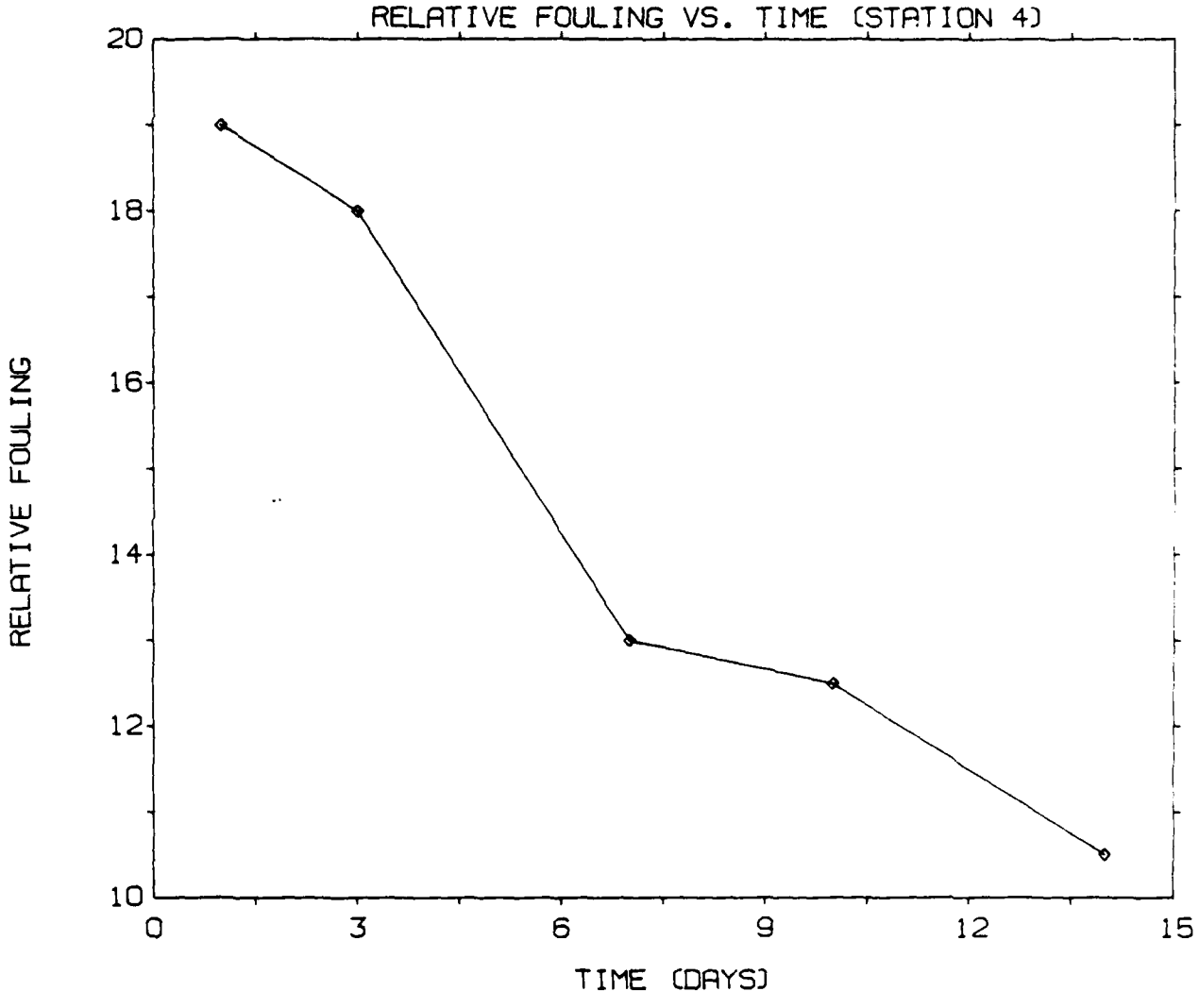
- FIGURE (41) -


RELATIVE FOULING VS. TIME (STATION 2)
RELATIVE FOULING
2
1.6
1.2
0.8
0.4
0
0
3
6
9
12
15
TIME (DAYS)

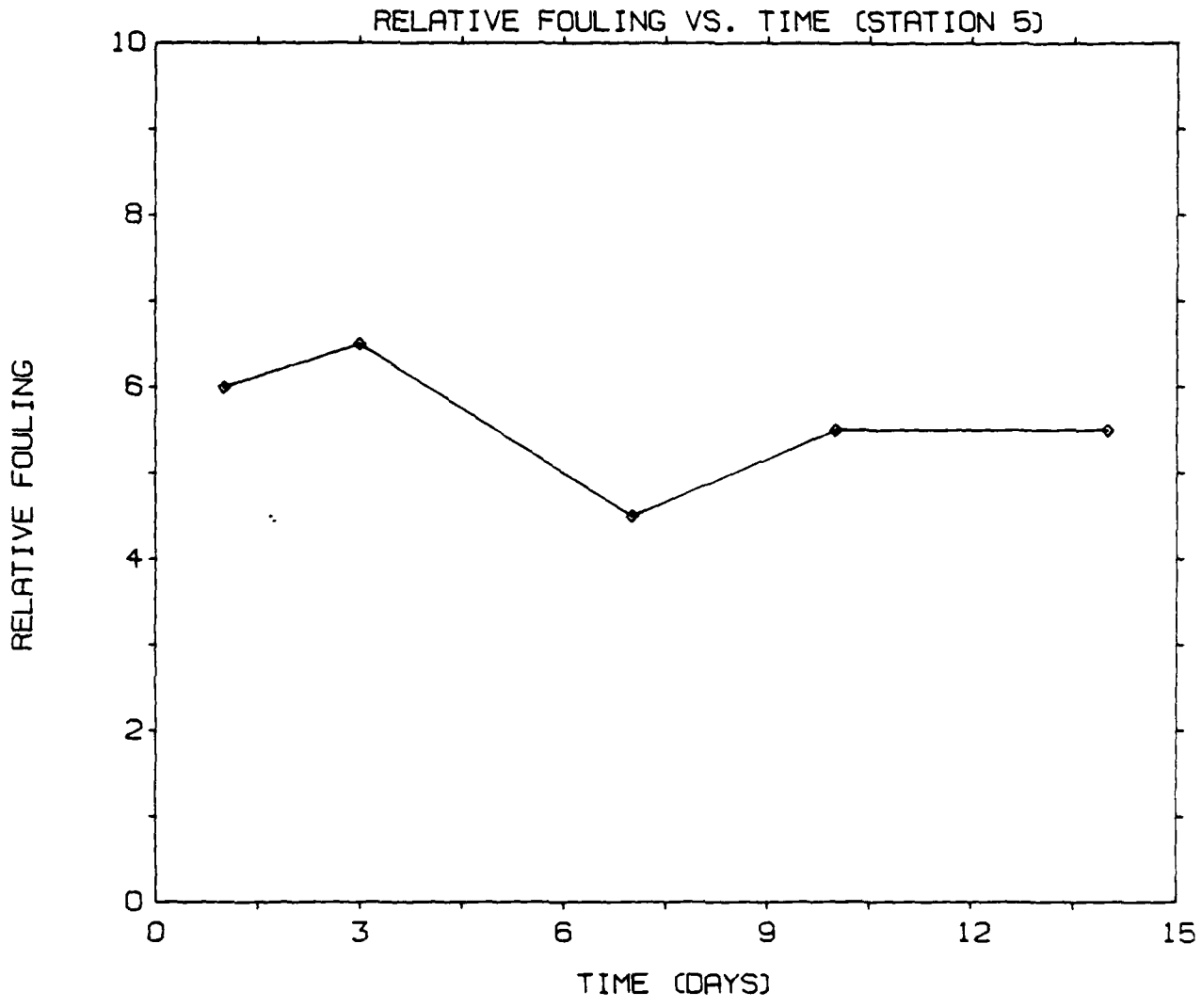
- FIGURE (42) -


RELATIVE FOULING VS. TIME (STATION 3)
RELATIVE FOULING
25
20
15
10
5
0
0
3
6
9
12
15
TIME (DAYS)

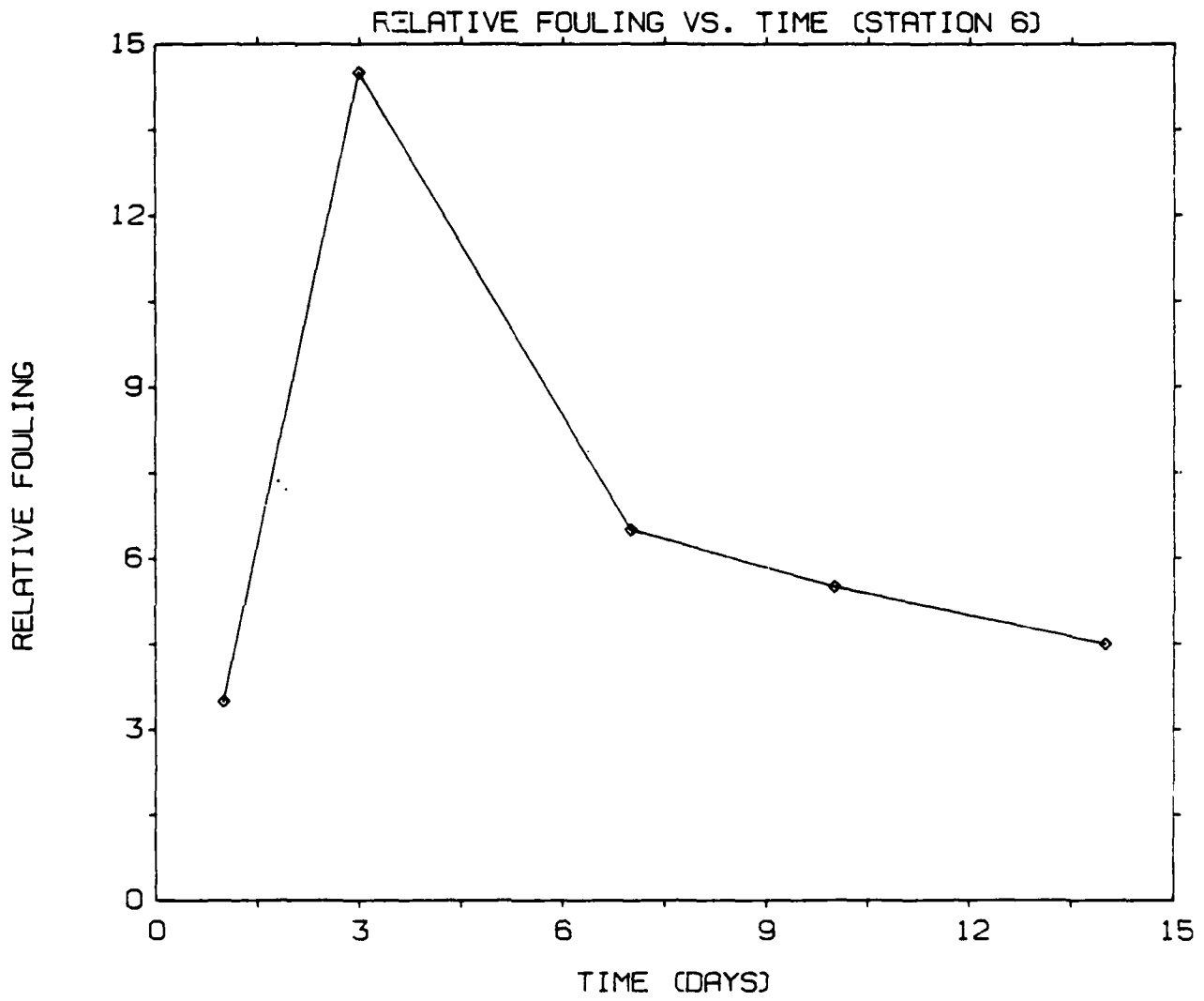
- FIGURE (43) -


RELATIVE FOULING VS. TIME (STATION 4)
RELATIVE FOULING
20
18
16
14
12
10
0
3
6
9
12
15
TIME (DAYS)

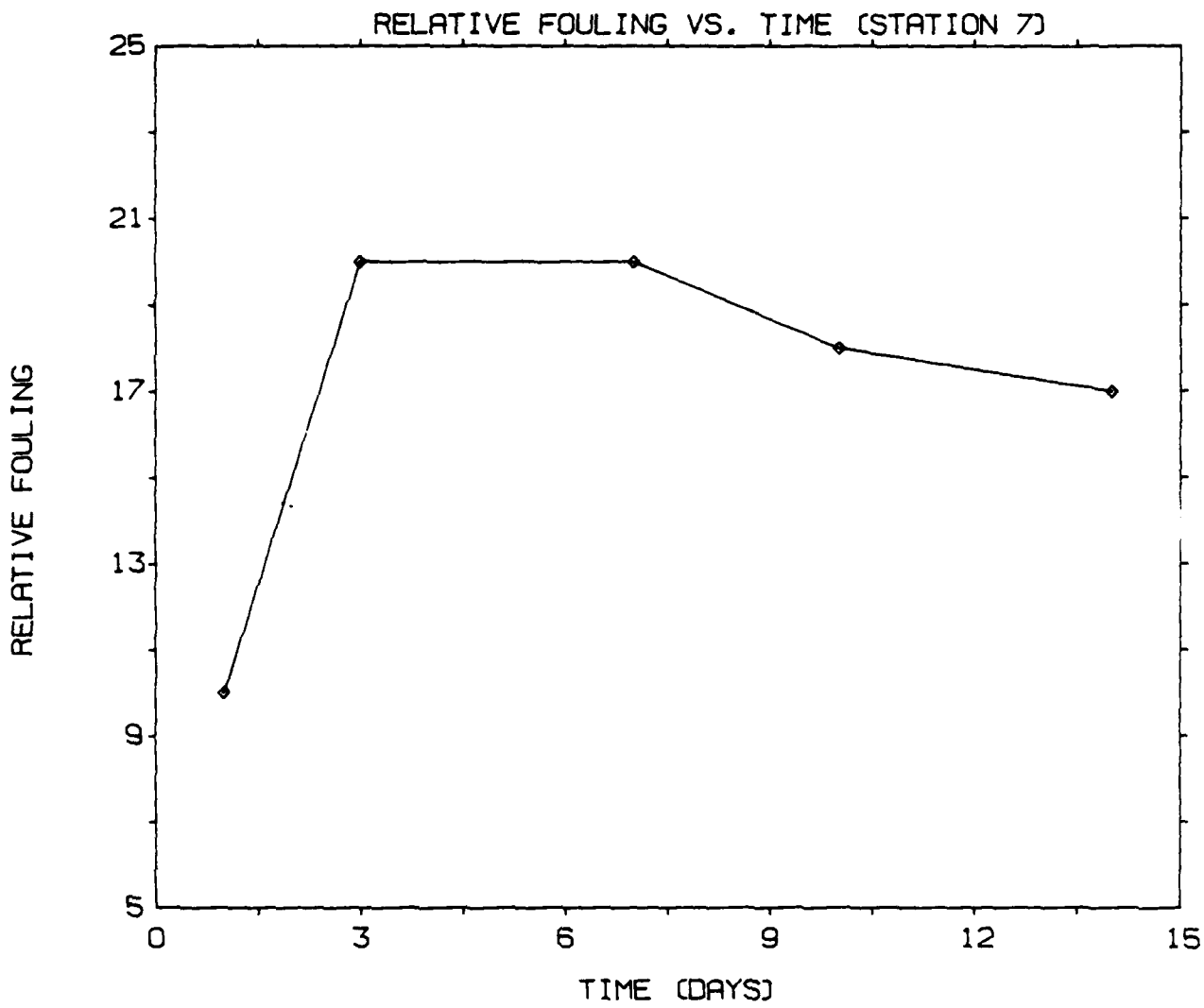
- FIGURE (44) -


RELATIVE FOULING VS. TIME (STATION 5)
RELATIVE FOULING
TIME (DAYS)
0
2
4
6
8
10
0
3
6
9
12
15

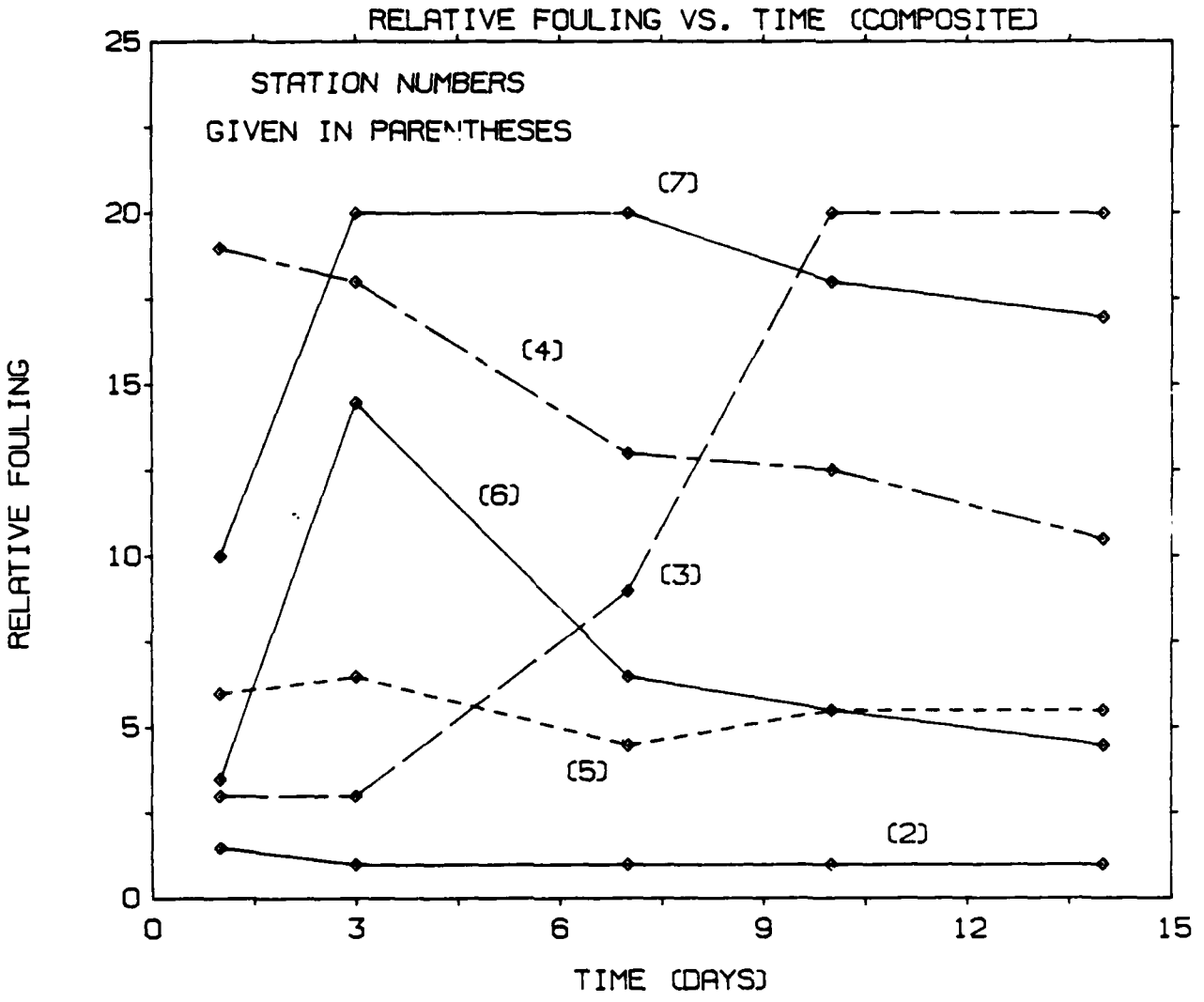
- FIGURE (45) -


RELATIVE FOULING VS. TIME (STATION 6)
RELATIVE FOULING
TIME (DAYS)
0
3
6
9
12
15

- FIGURE (46) -


RELATIVE FOULING VS. TIME (STATION 7)
RELATIVE FOULING
25
21
17
13
9
5
0
3
6
9
12
15
TIME (DAYS)

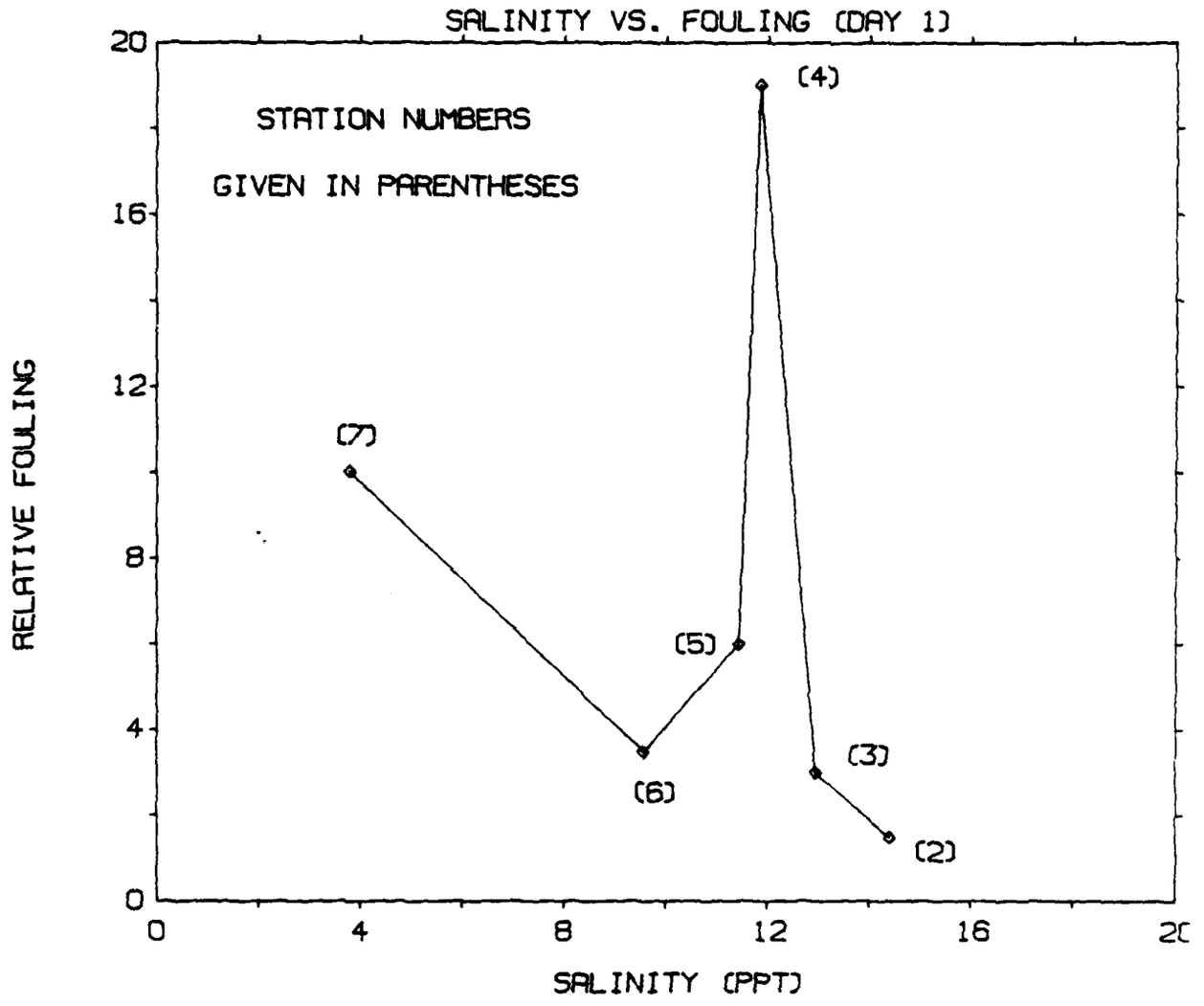
- FIGURE (47) -


RELATIVE FOULING VS. TIME (COMPOSITE)
STATION NUMBERS
GIVEN IN PARENTHESES
(7)
(4)
(6)
(3)
(5)
(2)
RELATIVE FOULING
TIME (DAYS)
0
5
10
15
20
25
0
3
6
9
12
15

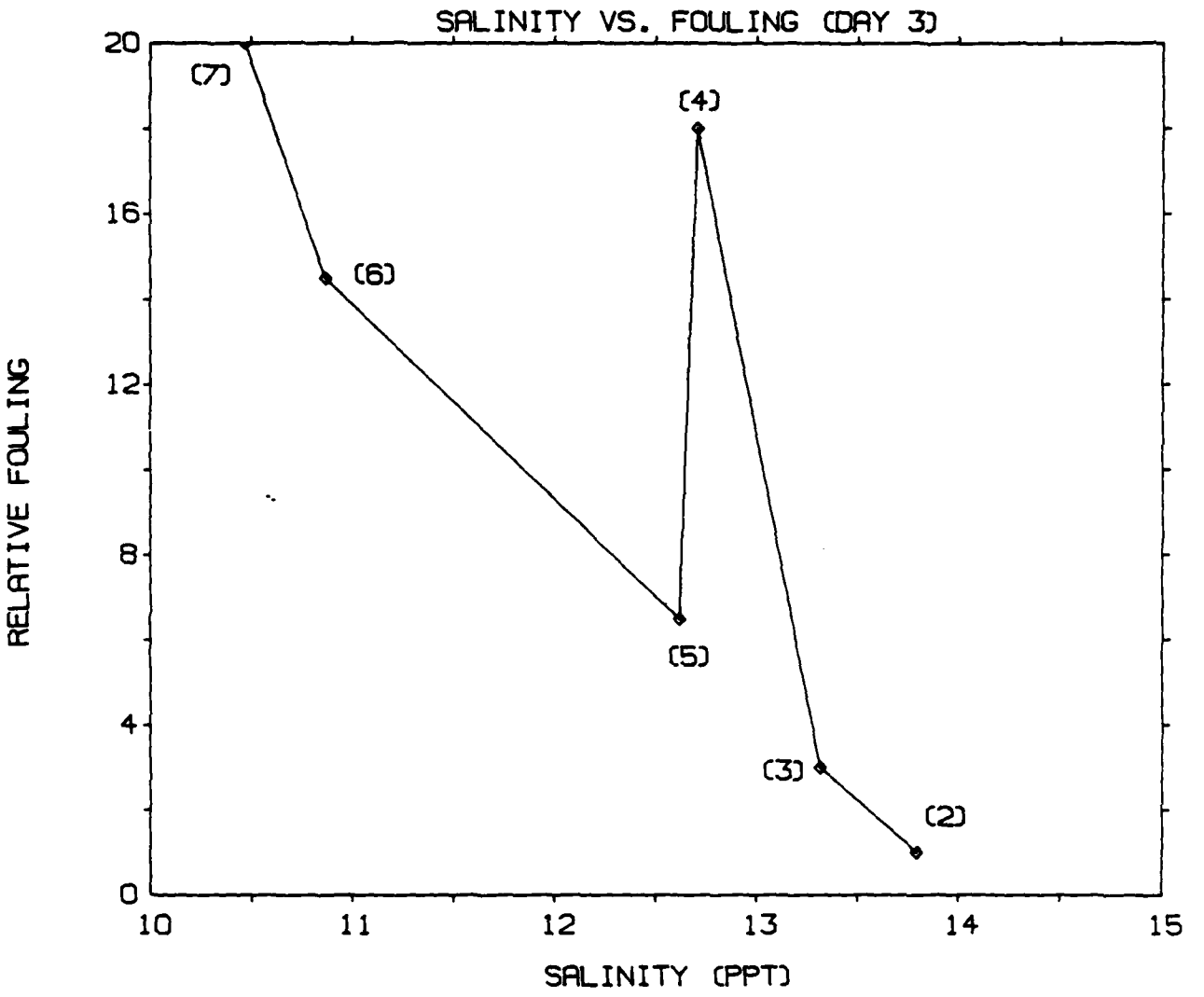
APPENDIX C - EXPERIMENT ONE:

GRAPHS OF WATER VARIABLES VS. RELATIVE FOULING

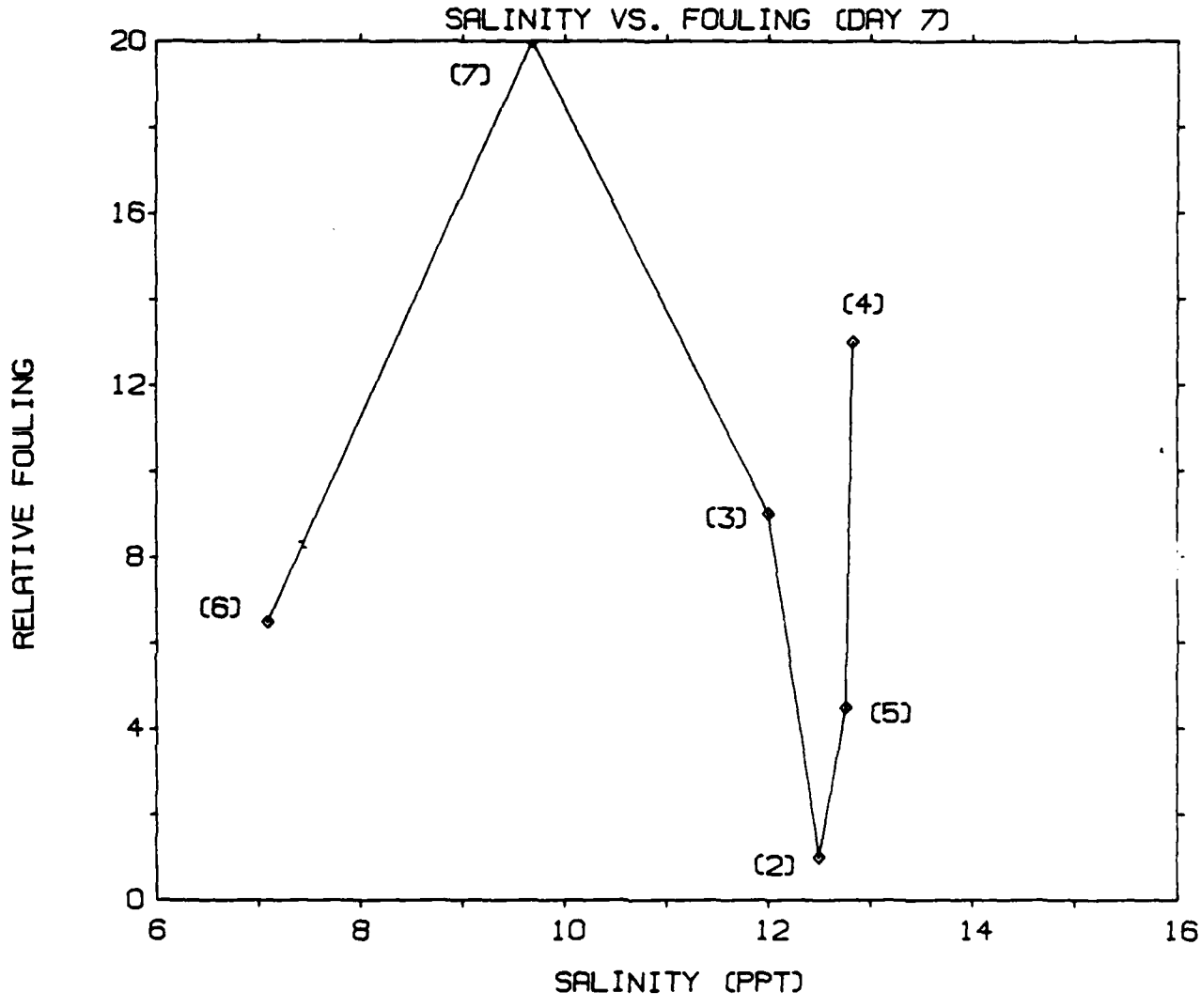
- FIGURE (48) -


SALINITY VS. FOULING (DAY 1)
STATION NUMBERS
GIVEN IN PARENTHESES
(4)
(7)
(5)
(3)
(6)
(2)
RELATIVE FOULING
SALINITY (PPT)
0
4
8
12
16
20

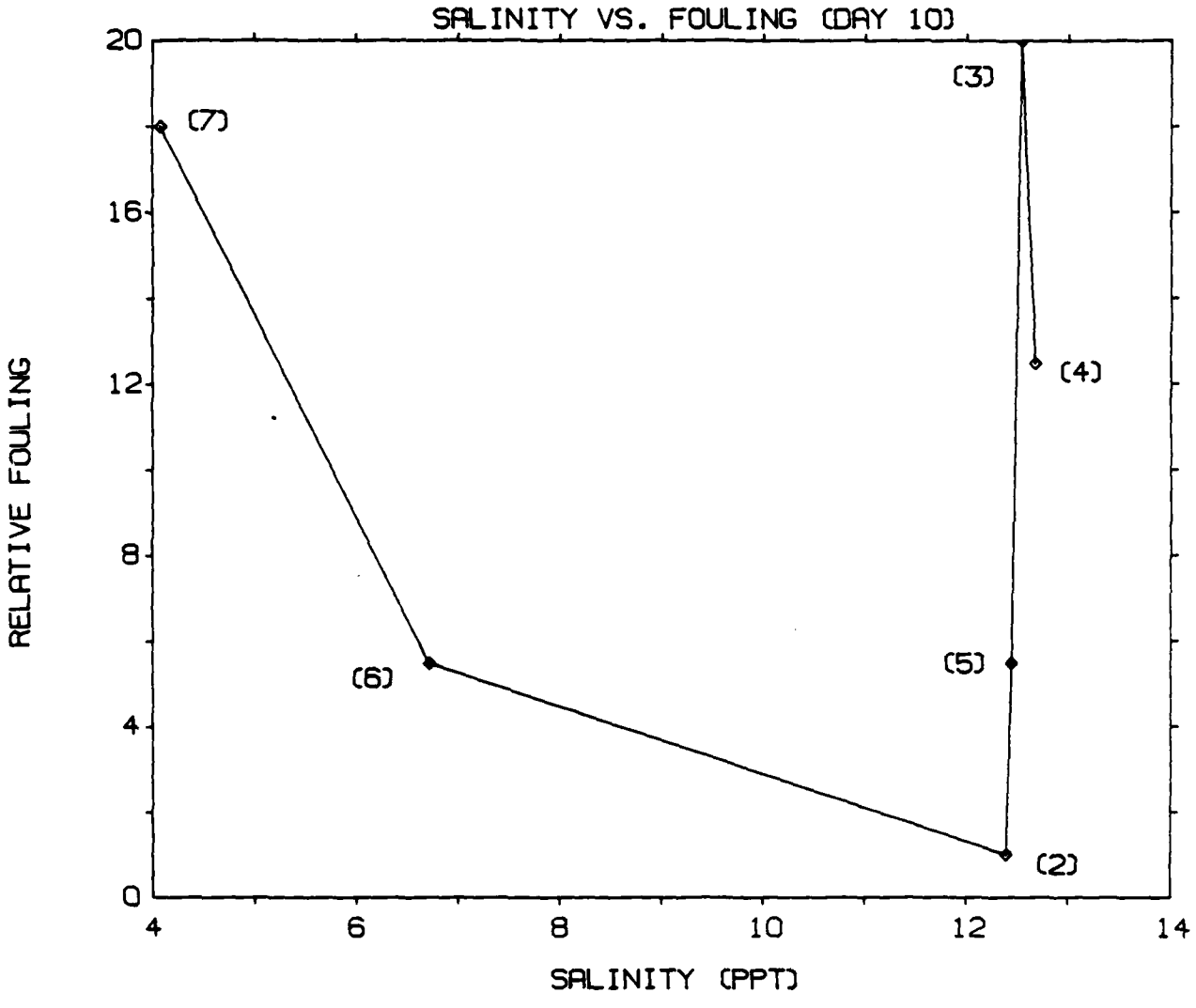
- FIGURE (49) -


SALINITY VS. FOULING (DAY 3)
RELATIVE FOULING
SALINITY (PPT)
20
16
12
8
4
0
10
11
12
13
14
15
(7)
(6)
(4)
(5)
(3)
(2)

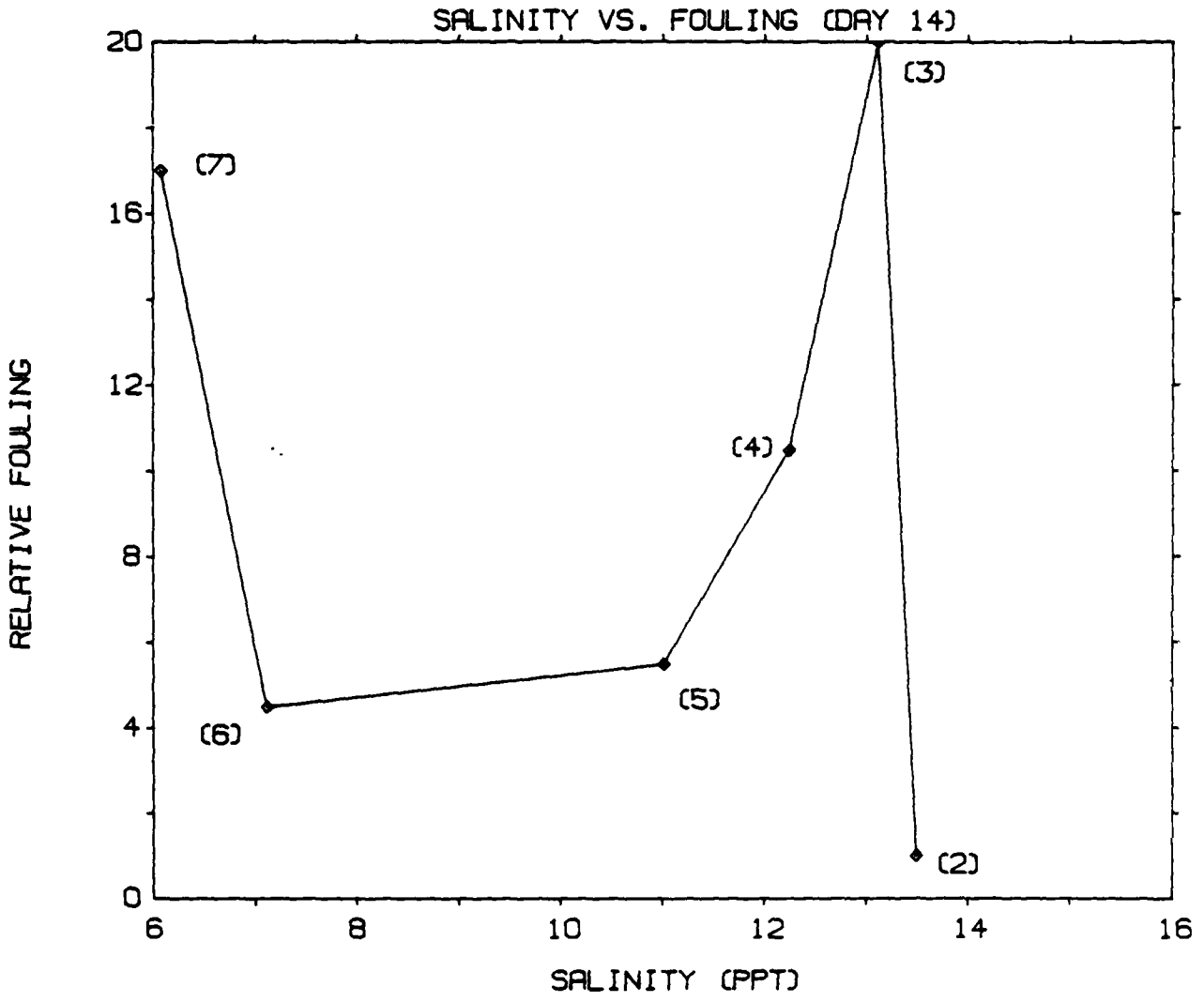
- FIGURE (50) -


SALINITY VS. FOULING (DAY 7)
RELATIVE FOULING
SALINITY (PPT)
0
4
8
12
16
20
6
8
10
12
14
16
(7)
(4)
(3)
(6)
(5)
(2)

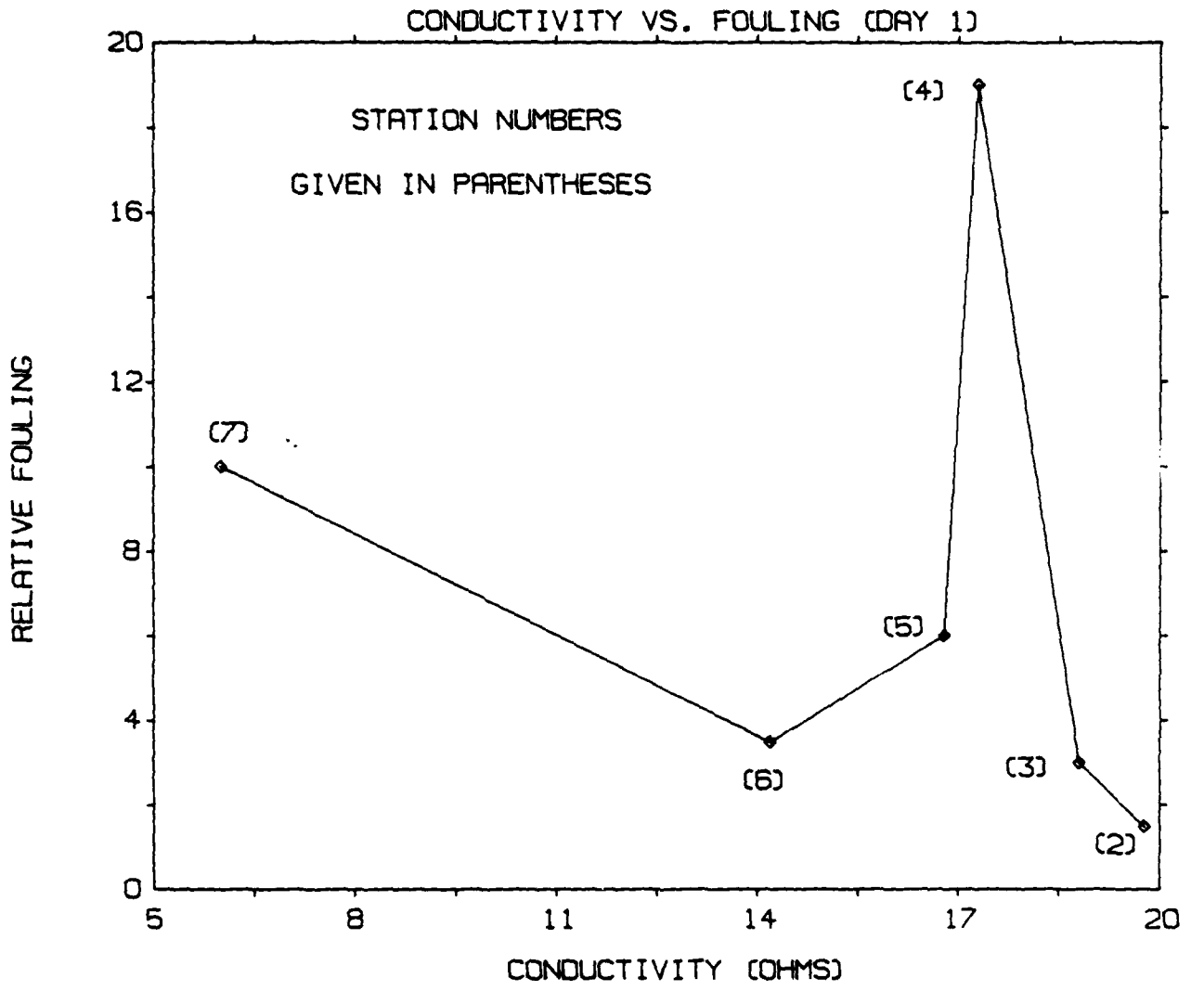
- FIGURE (51) -


SALINITY VS. FOULING (DAY 10)
RELATIVE FOULING
SALINITY (PPT)
0
4
8
12
16
20
4
6
8
10
12
14
(7)
(3)
(4)
(6)
(5)
(2)

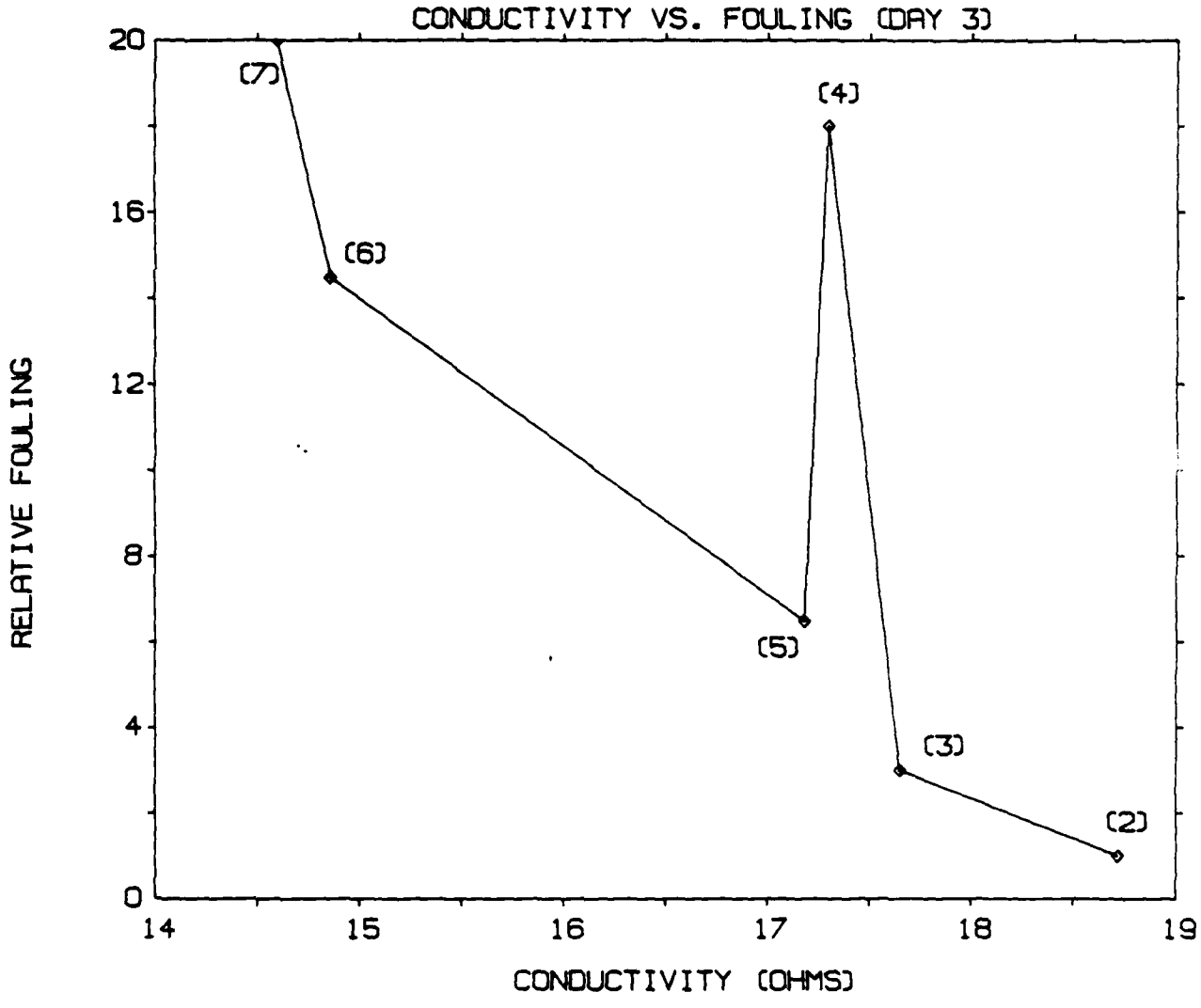
- FIGURE (52) -


SALINITY VS. FOULING (DAY 14)
RELATIVE FOULING
SALINITY (PPT)
0
4
8
12
16
20
6
8
10
12
14
16
(7)
(6)
(5)
(4)
(3)
(2)

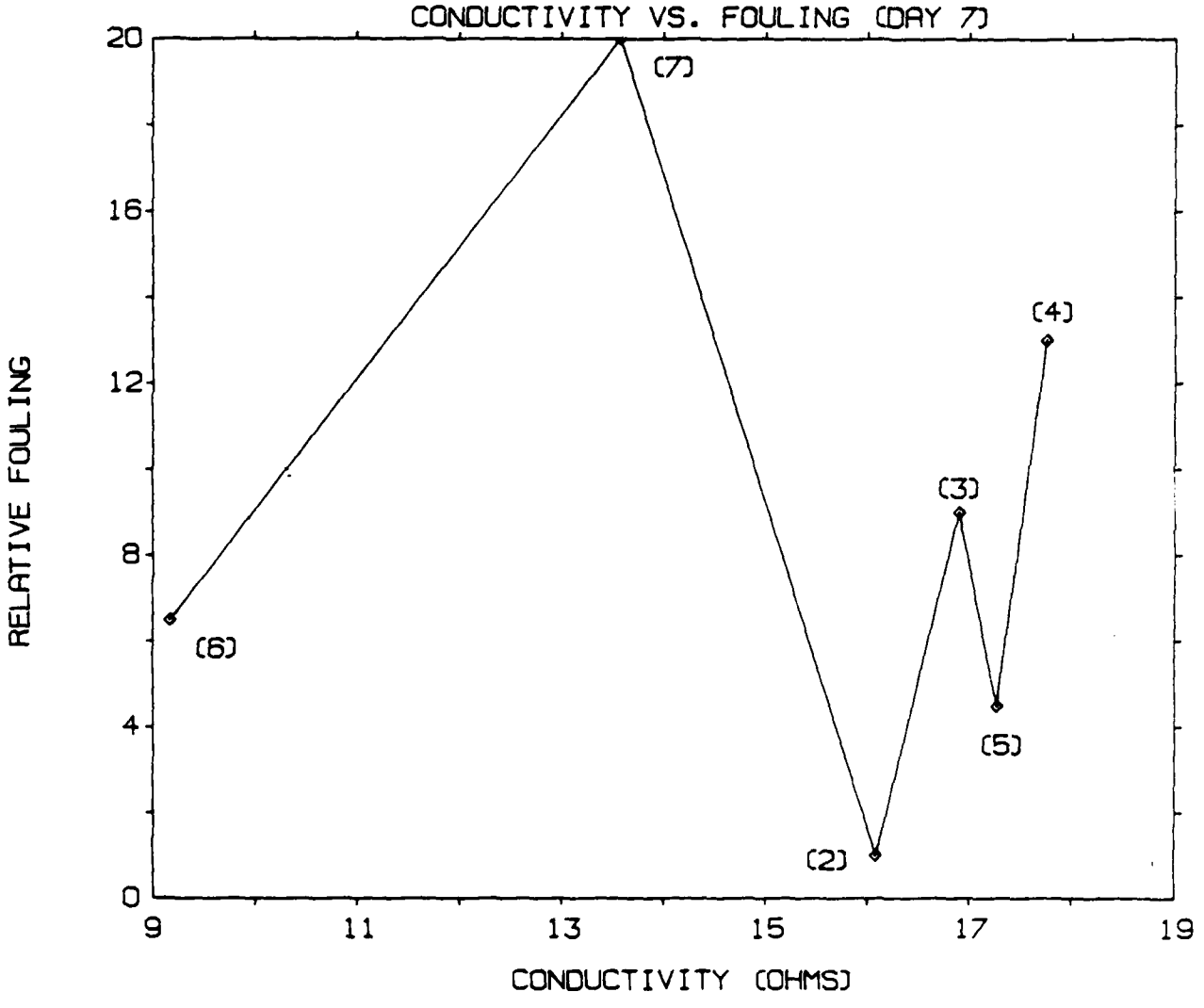
- FIGURE (53) -


CONDUCTIVITY VS. FOULING (DAY 1)
STATION NUMBERS
GIVEN IN PARENTHESES
(4)
(7)
(5)
(6)
(3)
(2)
RELATIVE FOULING
CONDUCTIVITY (OHMS)
0
4
8
12
16
20
5
8
11
14
17
20

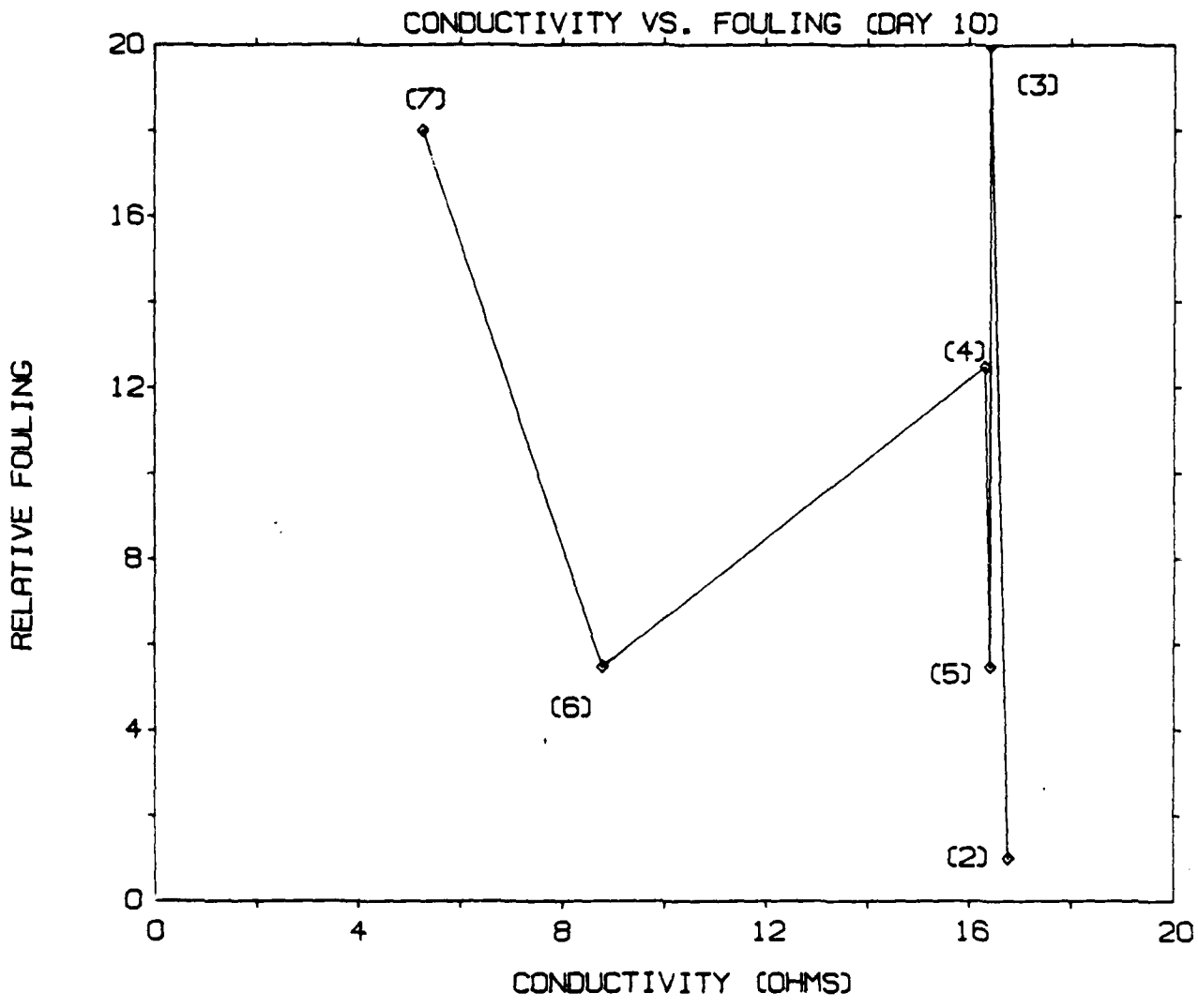
- FIGURE (54) -


CONDUCTIVITY VS. FOULING (DAY 3)
RELATIVE FOULING
CONDUCTIVITY (OHMS)
20
16
12
8
4
0
14
15
16
17
18
19
(7)
(6)
(5)
(4)
(3)
(2)

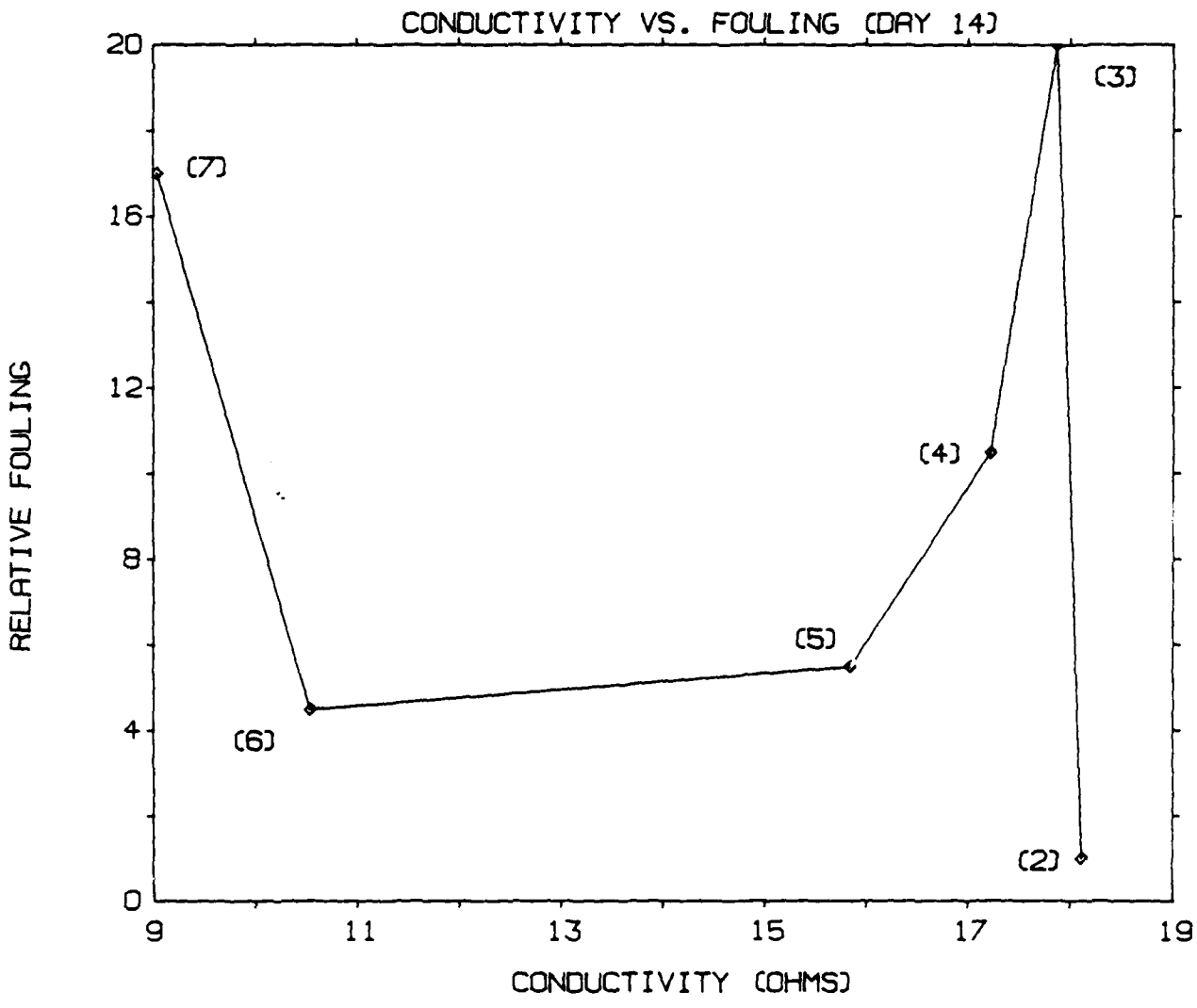
- FIGURE (55) -


CONDUCTIVITY VS. FOULING (DAY 7)
RELATIVE FOULING
CONDUCTIVITY (OHMS)
0
4
8
12
16
20
9
11
13
15
17
19
(7)
(6)
(2)
(3)
(4)
(5)

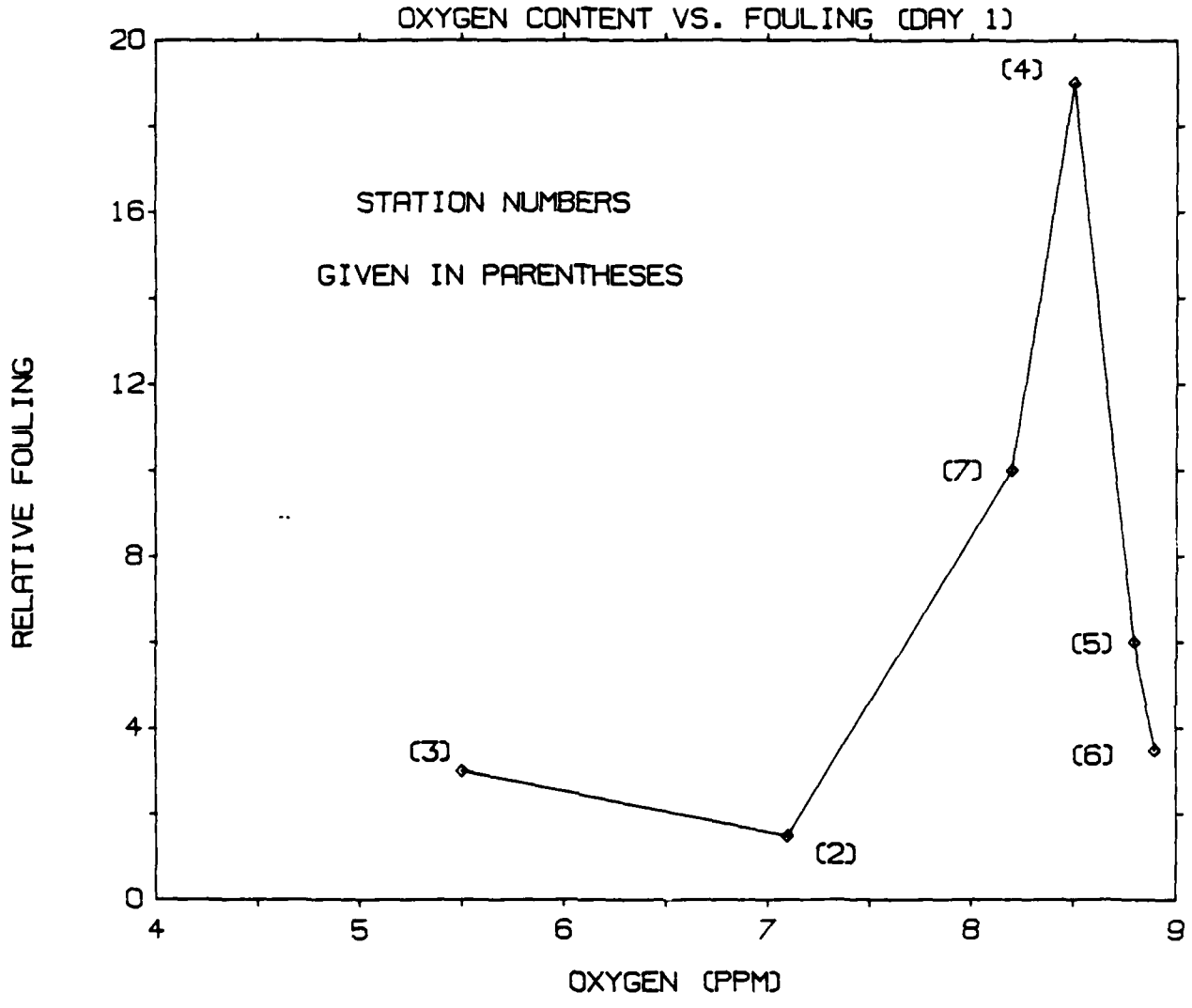
- FIGURE (56) -


CONDUCTIVITY VS. FOULING (DAY 10)
RELATIVE FOULING
CONDUCTIVITY (OHMS)
0
4
8
12
16
20
(7)
(3)
(4)
(6)
(5)
(2)

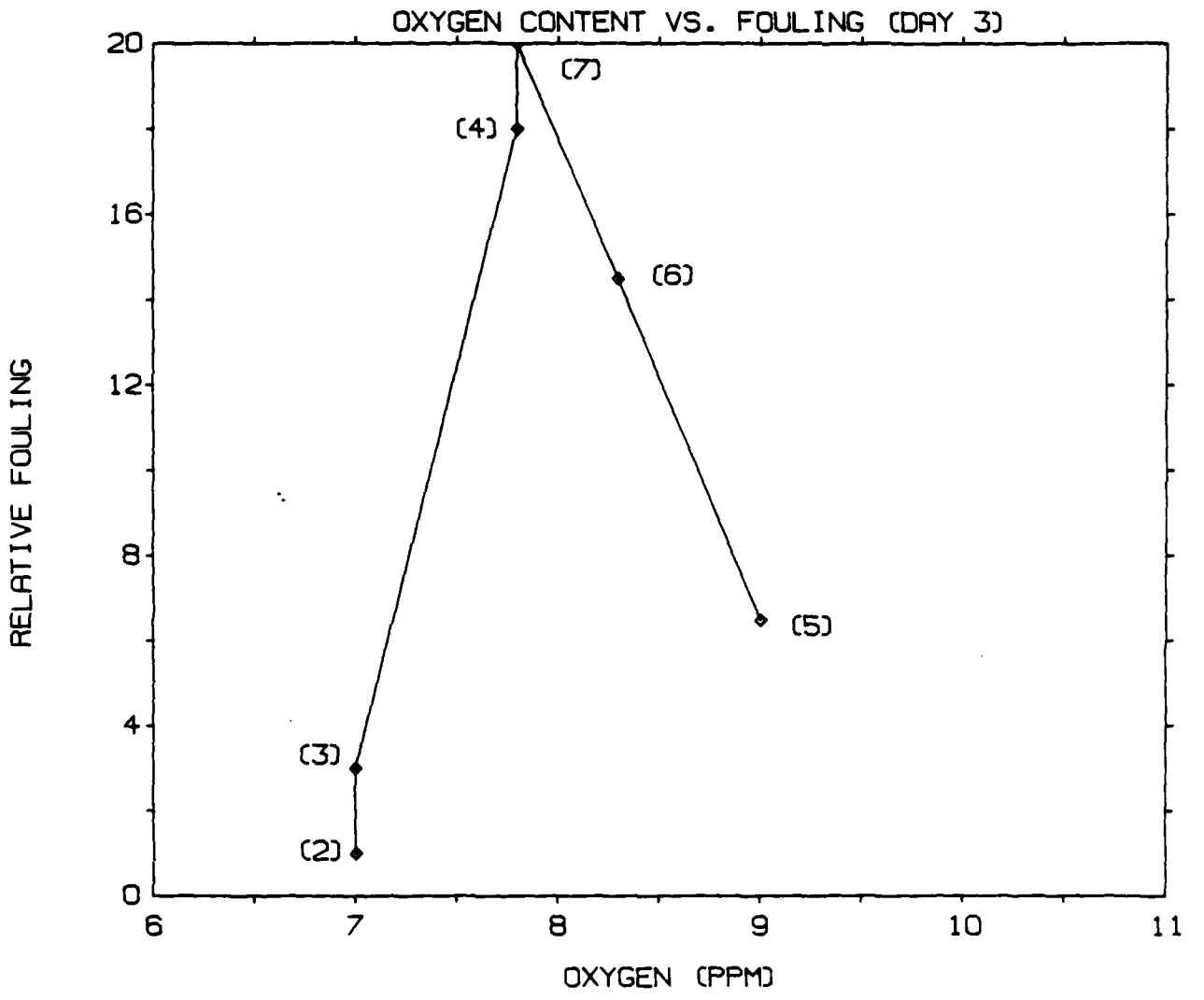
- FIGURE (57) -


CONDUCTIVITY VS. FOULING (DAY 14)
RELATIVE FOULING
CONDUCTIVITY (OHMS)
0
4
8
12
16
20
9
11
13
15
17
19
(7)
(6)
(5)
(4)
(3)
(2)

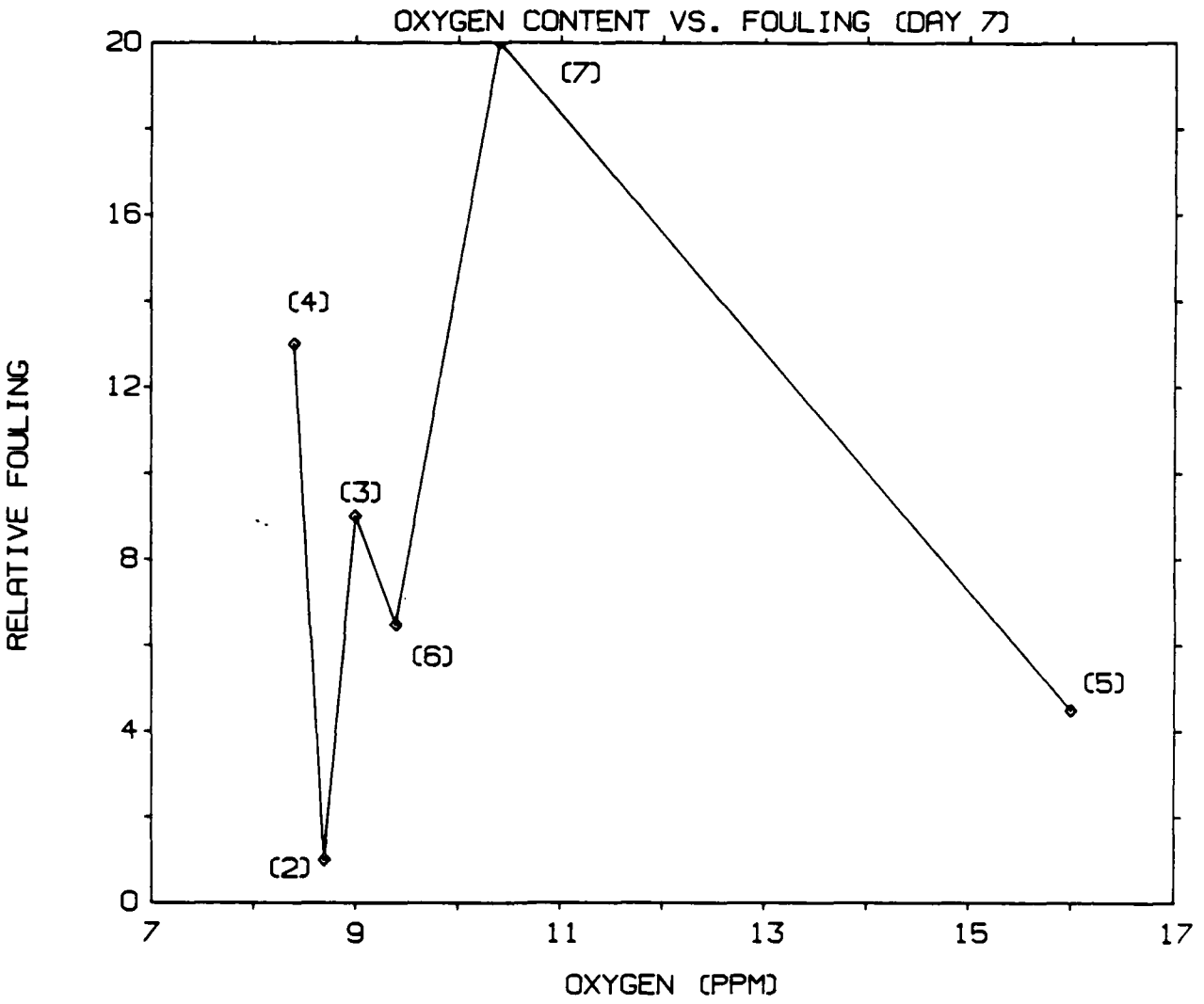
- FIGURE (58) -


OXYGEN CONTENT VS. FOULING (DAY 1)
STATION NUMBERS
GIVEN IN PARENTHESES
RELATIVE FOULING
OXYGEN (PPM)
0
4
8
12
16
20
4
5
6
7
8
9
(4)
(7)
(5)
(6)
(3)
(2)

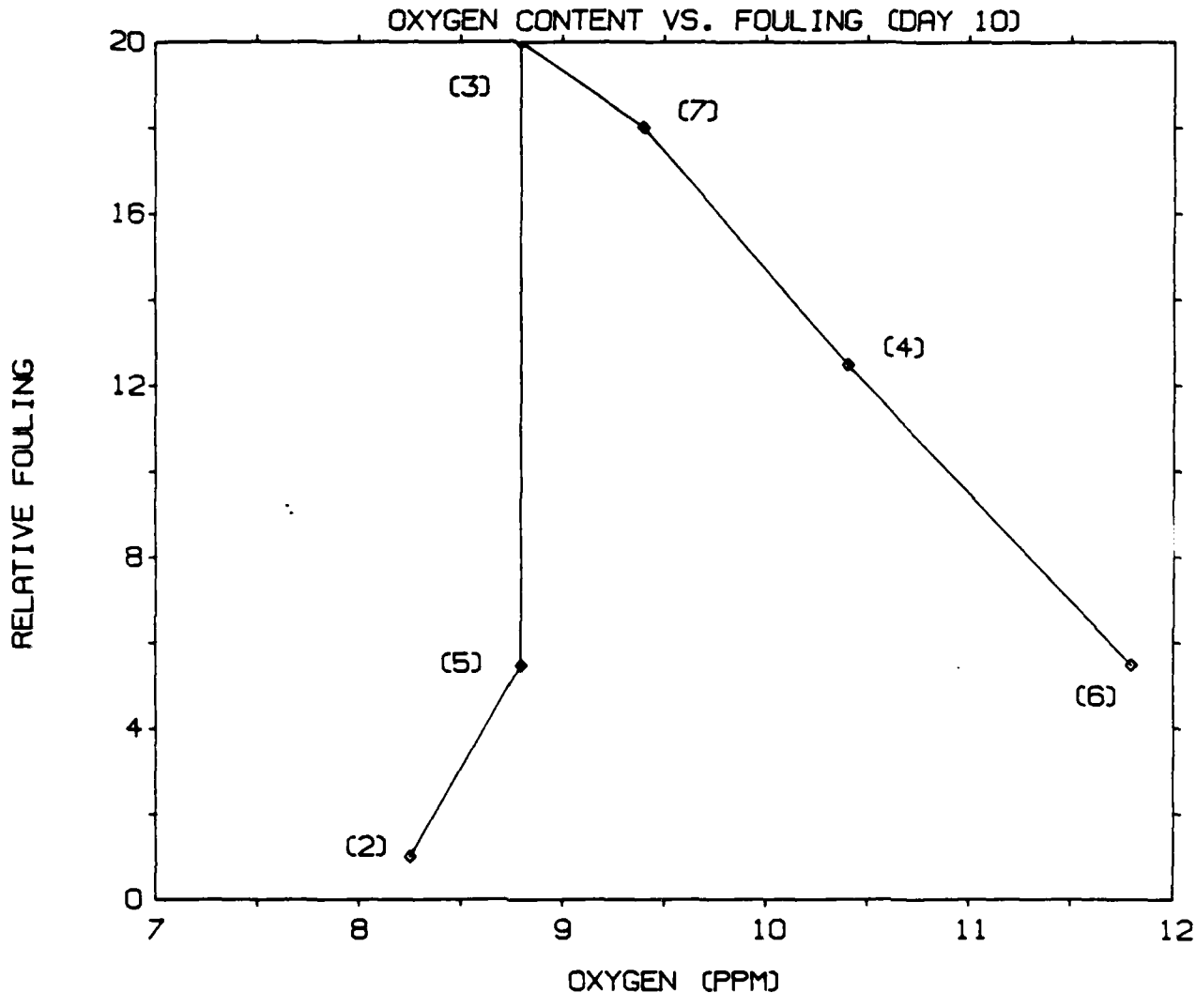
- FIGURE (59) -


OXYGEN CONTENT VS. FOULING (DAY 3)
RELATIVE FOULING
OXYGEN (PPM)
0
4
8
12
16
20
6
7
8
9
10
11
(2)
(3)
(4)
(5)
(6)
(7)

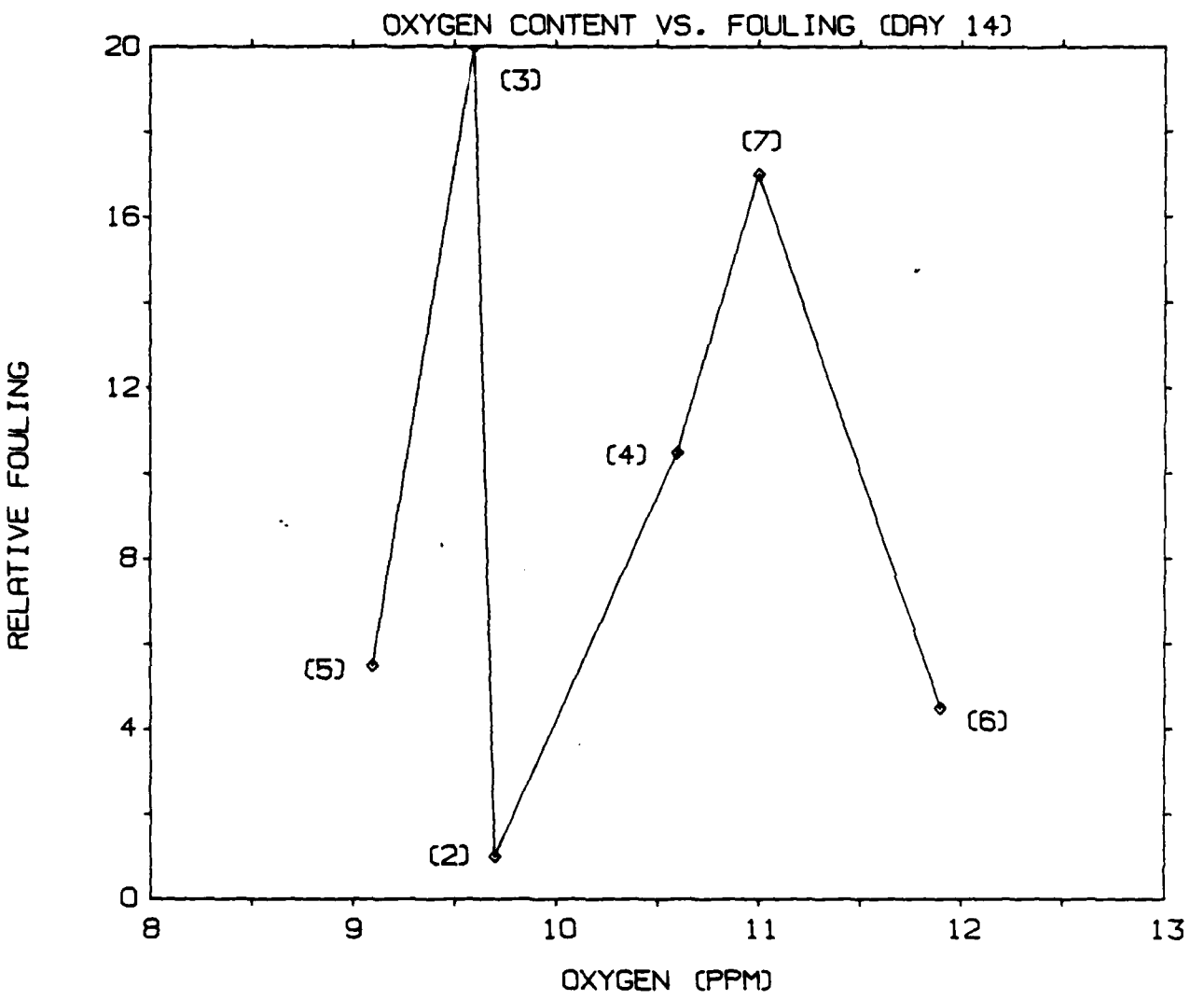
- FIGURE (60) -


OXYGEN CONTENT VS. FOULING (DAY 7)
RELATIVE FOULING
OXYGEN (PPM)
0
4
8
12
16
20
7
9
11
13
15
17
(2)
(3)
(4)
(5)
(6)
(7)

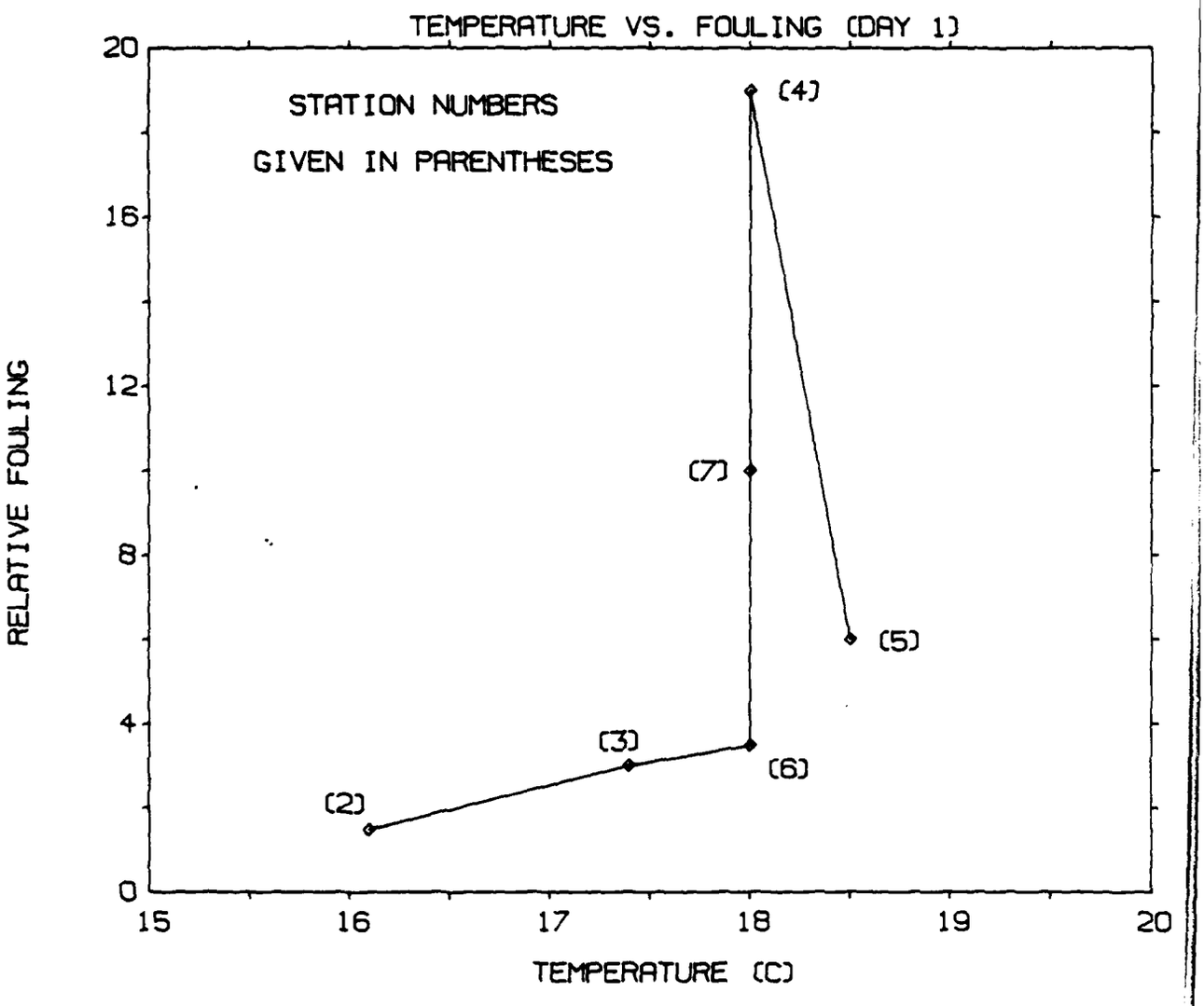
- FIGURE (61) -


OXYGEN CONTENT VS. FOULING (DAY 10)
RELATIVE FOULING
OXYGEN (PPM)
0
4
8
12
16
20
7
8
9
10
11
12
(2)
(3)
(4)
(5)
(6)
(7)

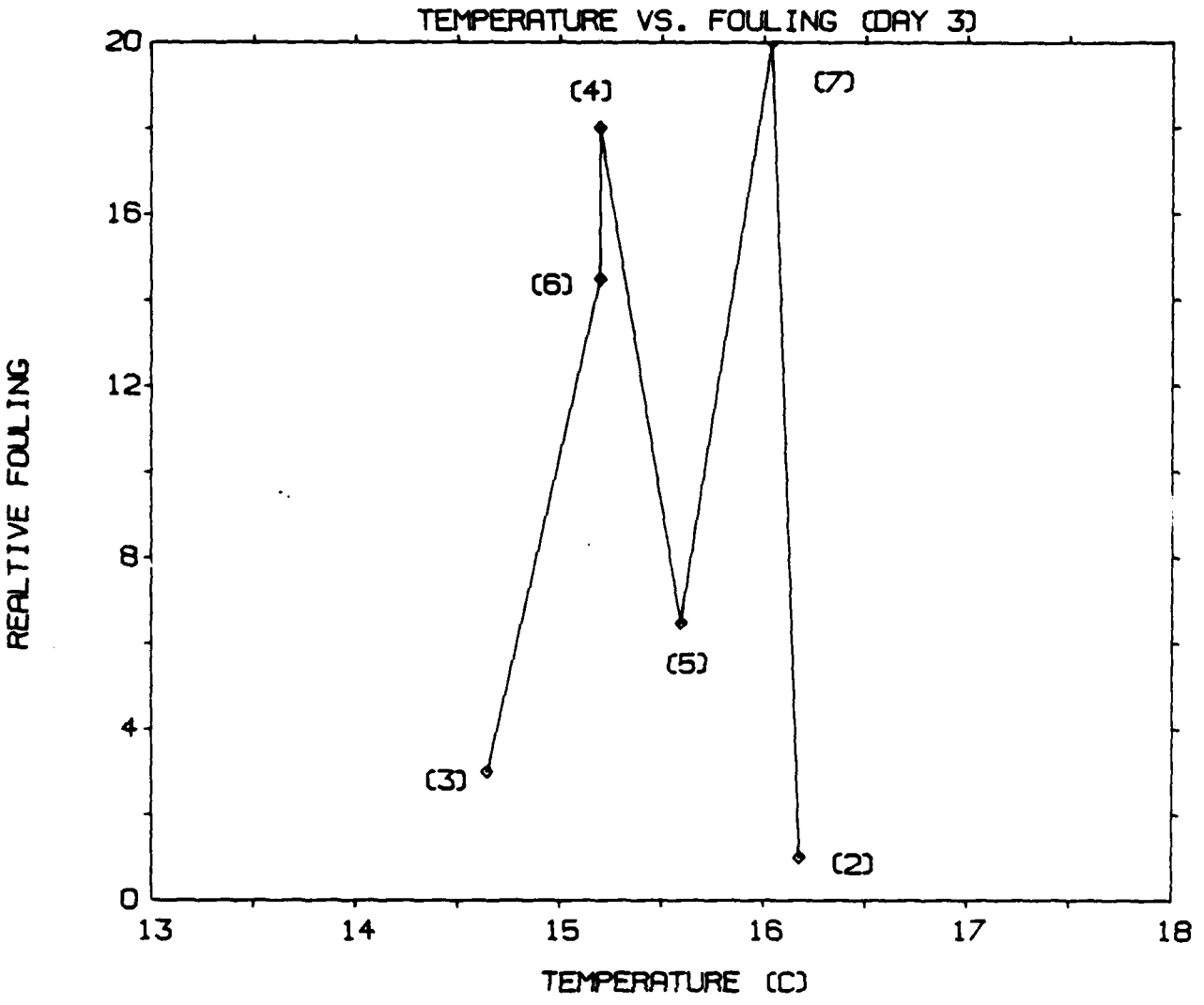
- FIGURE (62) -


OXYGEN CONTENT VS. FOULING (DAY 14)
RELATIVE FOULING
OXYGEN (PPM)
0
4
8
12
16
20
8
9
10
11
12
13
(1)
(2)
(3)
(4)
(5)
(6)
(7)

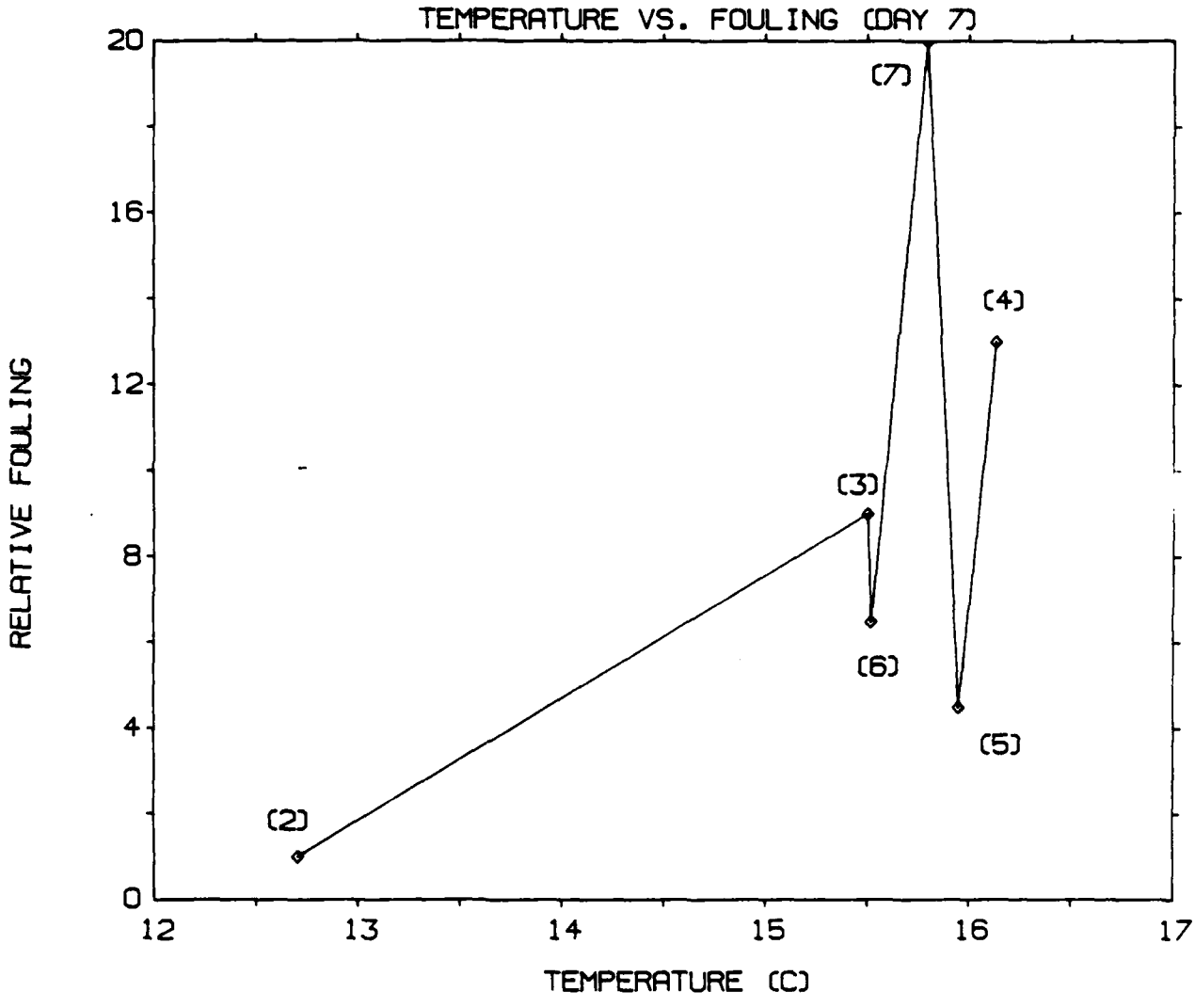
- FIGURE (63) -


TEMPERATURE VS. FOULING (DAY 1)
STATION NUMBERS
GIVEN IN PARENTHESES
(4)
(7)
(5)
(3)
(6)
(2)
RELATIVE FOULING
TEMPERATURE (C)
0
4
8
12
16
20
15
16
17
18
19
20

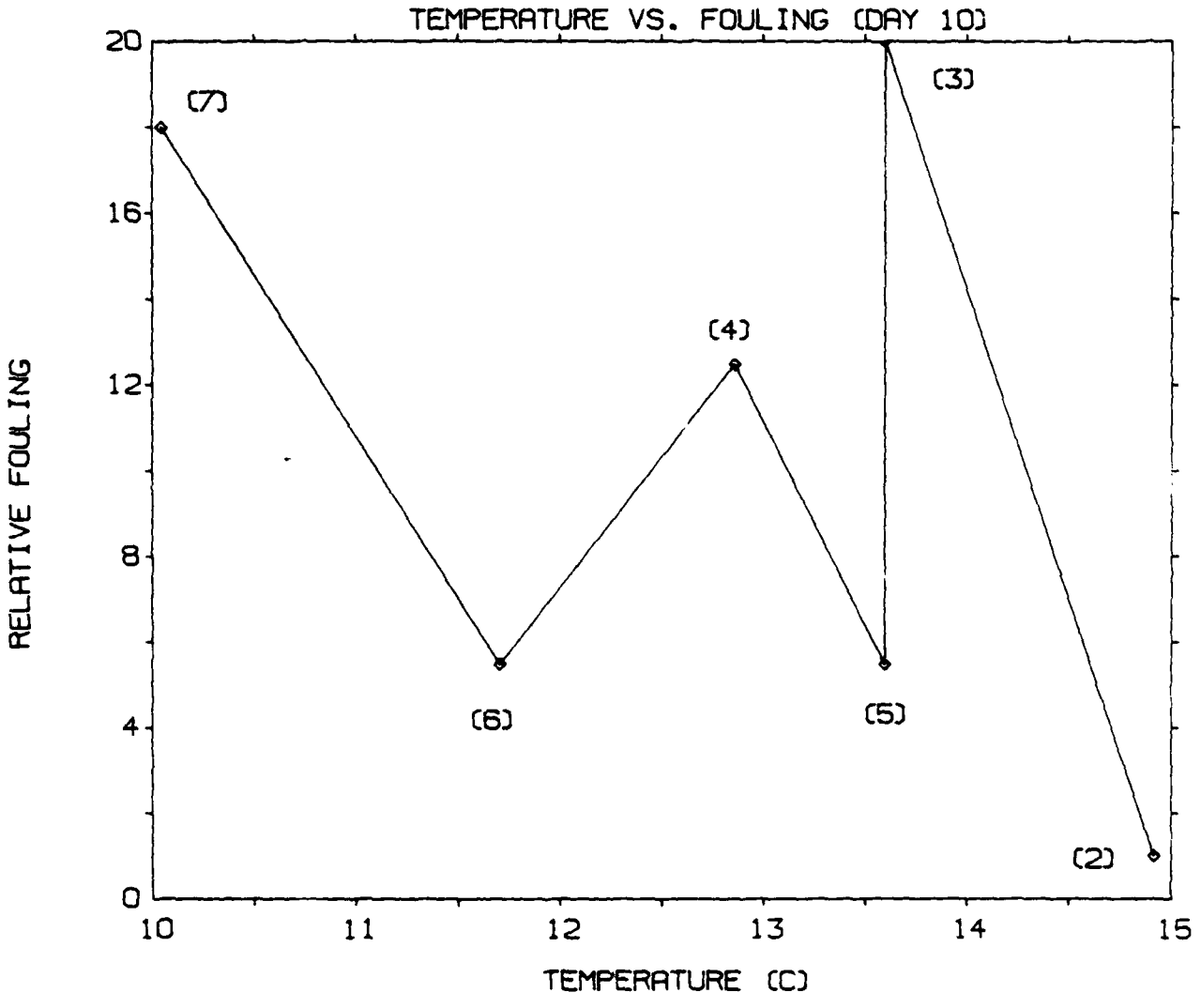
- FIGURE (64) -


TEMPERATURE VS. FOULING (DAY 3)
REALTIVE FOULING
TEMPERATURE (C)
0
4
8
12
16
20
13
14
15
16
17
18
(2)
(3)
(4)
(5)
(6)
(7)

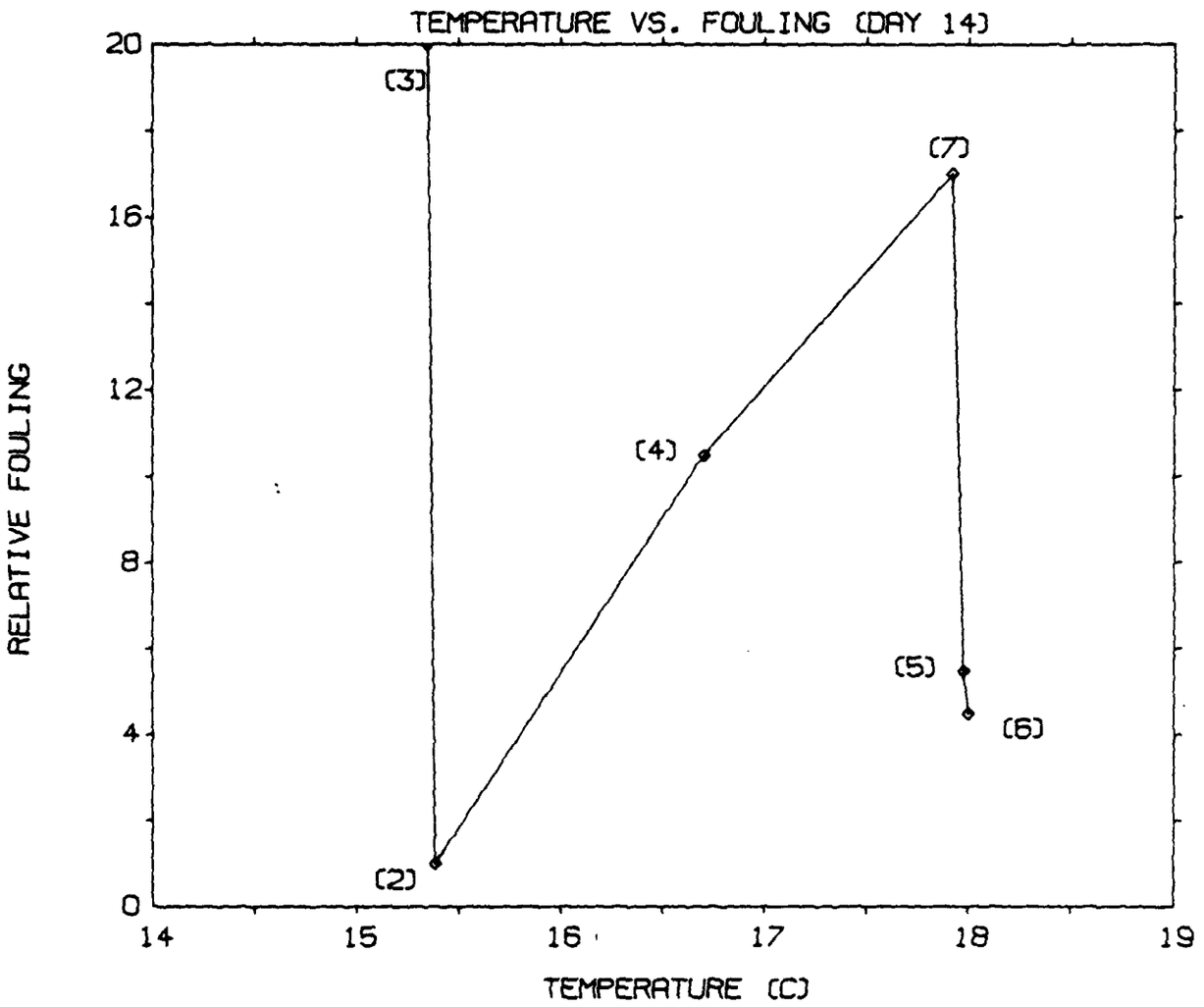
- FIGURE (65) -


TEMPERATURE VS. FOULING (DAY 7)
RELATIVE FOULING
TEMPERATURE (C)
0
4
8
12
16
20
12
13
14
15
16
17
(2)
(3)
(4)
(5)
(6)
(7)

- FIGURE (66) -


TEMPERATURE VS. FOULING (DAY 10)
RELATIVE FOULING
TEMPERATURE (C)
0
4
8
12
16
20
10
11
12
13
14
15
(7)
(6)
(4)
(5)
(3)
(2)

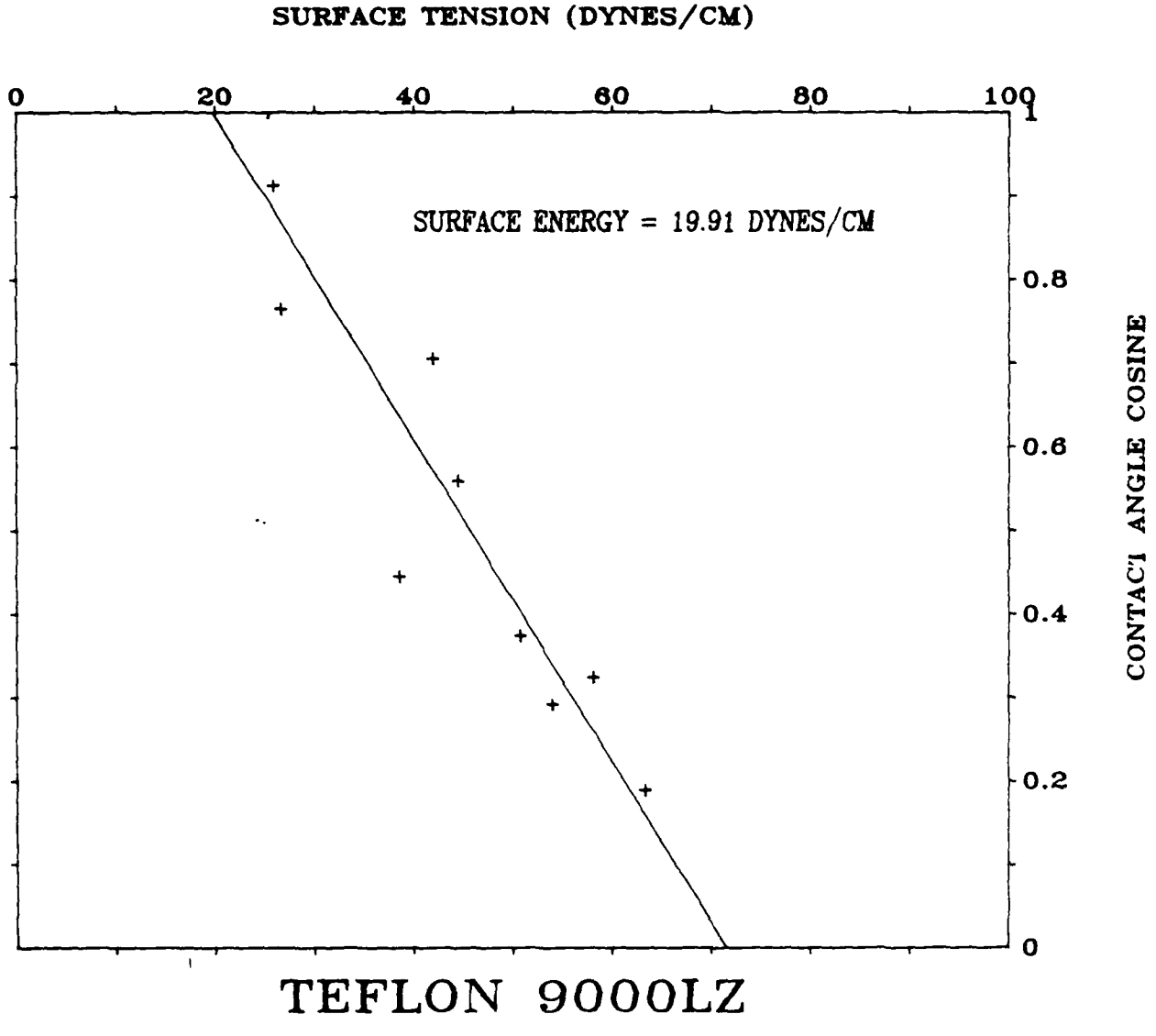
- FIGURE (67) -


TEMPERATURE VS. FOULING (DAY 14)
RELATIVE FOULING
TEMPERATURE (C)
0
4
8
12
16
20
14
15
16
17
18
19
(2)
(3)
(4)
(5)
(6)
(7)

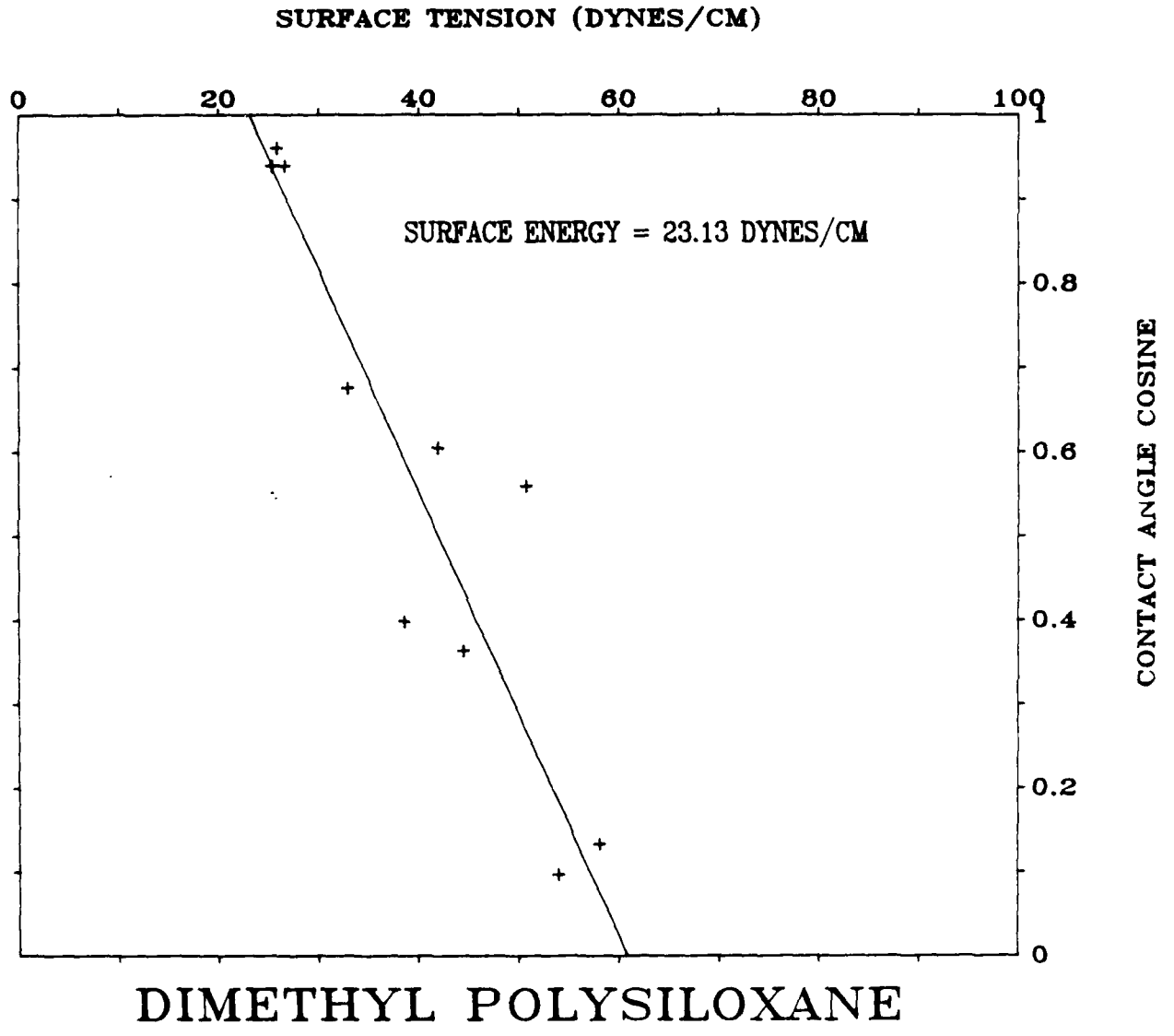
APPENDIX D - EXPERIMENT TWO:

GRAPHS OF LIQUID SURFACE TENSION VS. CONTACT ANGLE COSINE

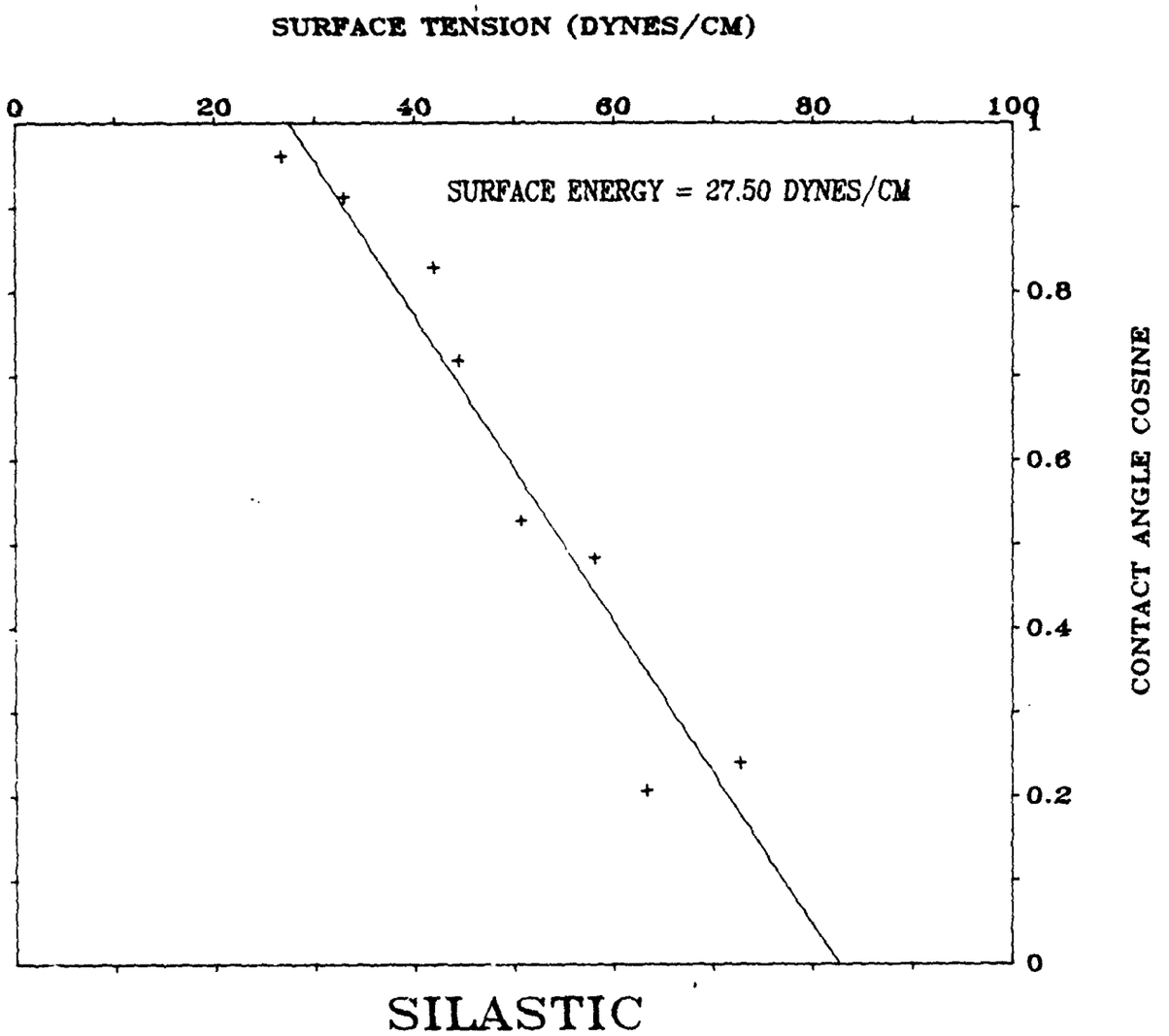
- FIGURE (68) -


SURFACE TENSION (DYNES/CM)
0
20
40
60
80
100
SURFACE ENERGY = 19.91 DYNES/CM
1
0.8
0.6
0.4
0.2
0
CONTACT ANGLE COSINE
TEFLON 9000LZ

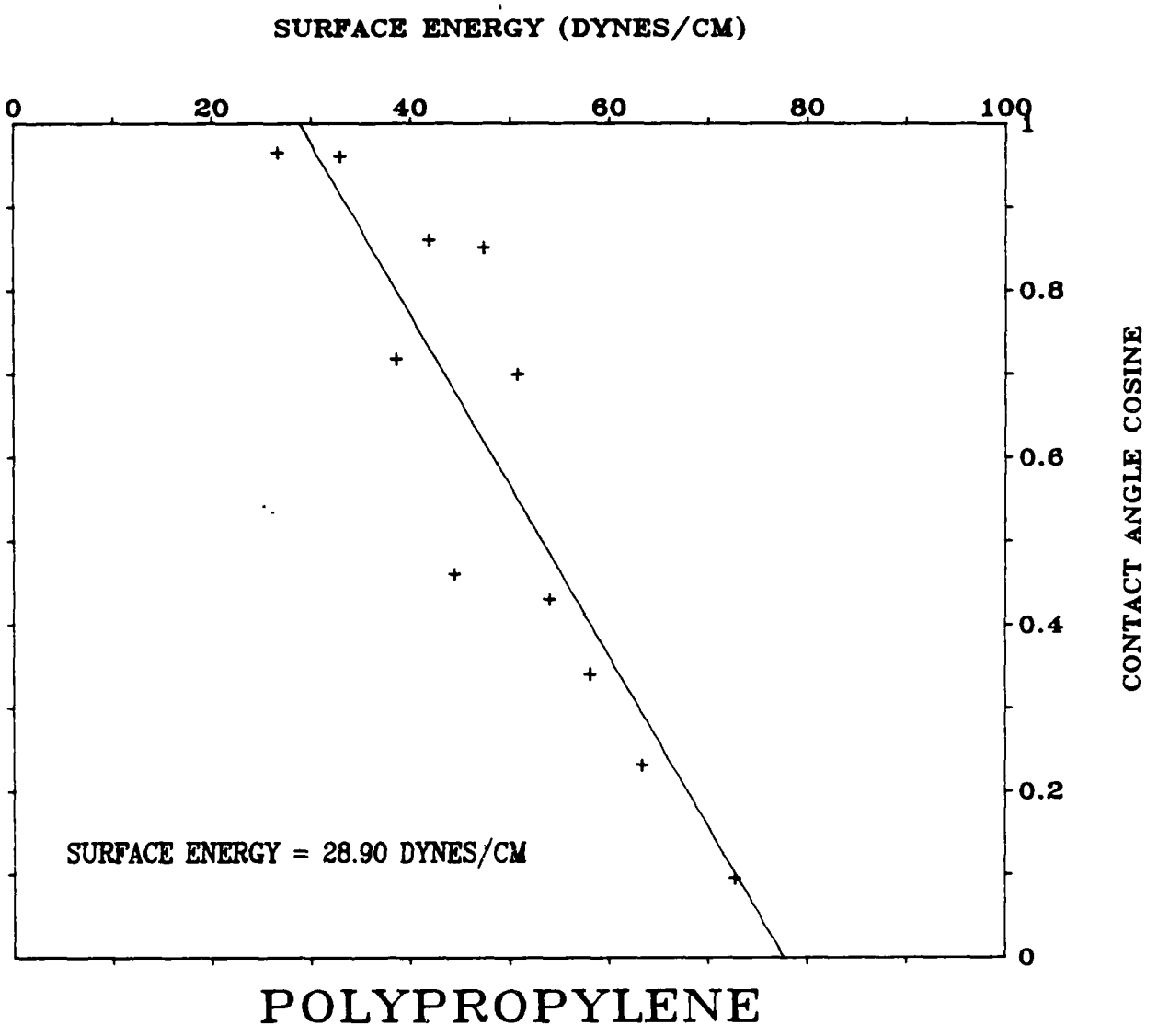
- FIGURE (69) -


SURFACE TENSION (DYNES/CM)
0
20
40
60
80
100
SURFACE ENERGY = 23.13 DYNES/CM
1
0.8
0.6
0.4
0.2
0
CONTACT ANGLE COSINE
DIMETHYL POLYSILOXANE

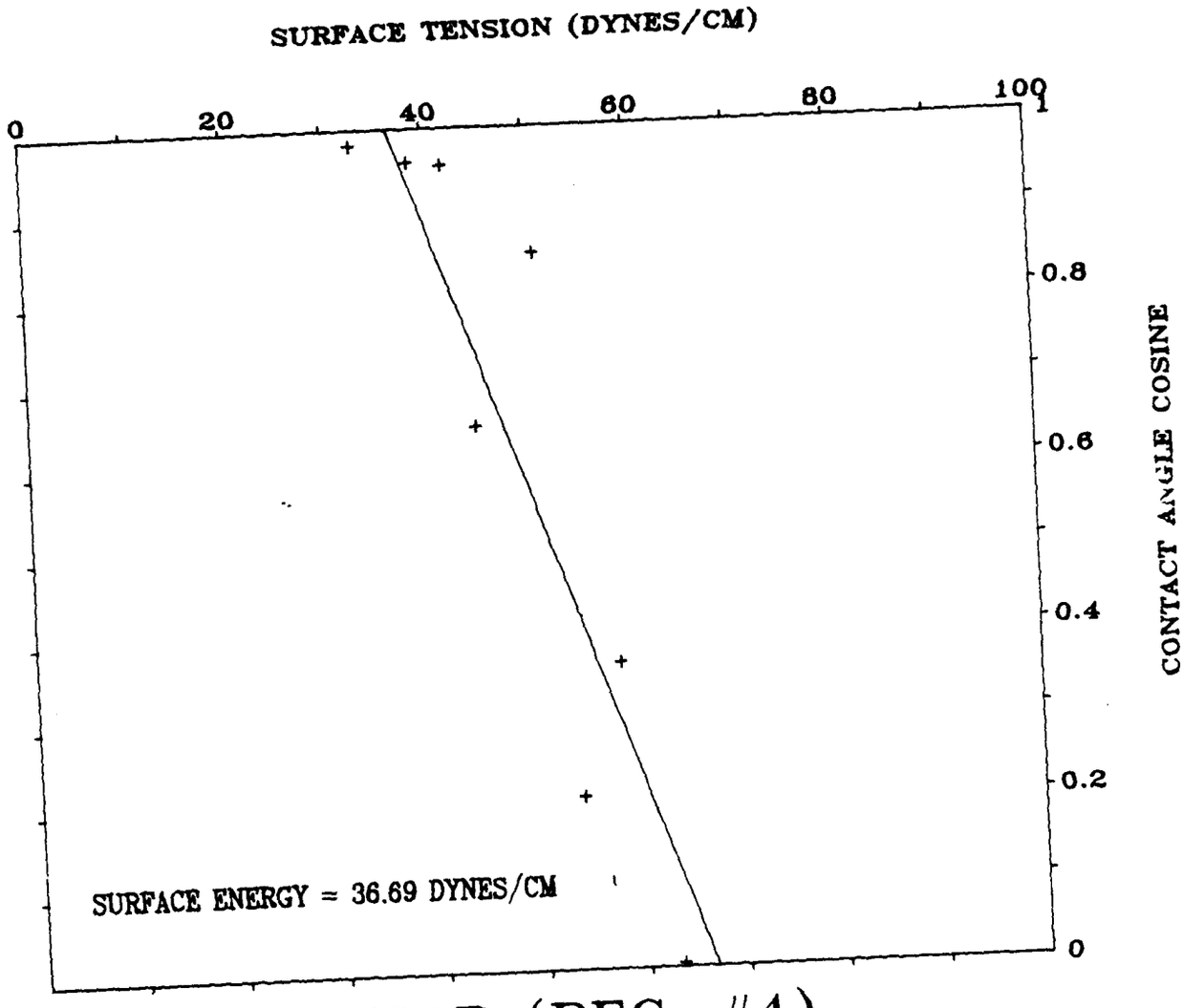
- FIGURE (70) -


SURFACE TENSION (DYNES/CM)
0
20
40
60
80
100
SURFACE ENERGY = 27.50 DYNES/CM
1
0.8
0.6
0.4
0.2
0
CONTACT ANGLE COSINE
SILASTIC

- FIGURE (71) -

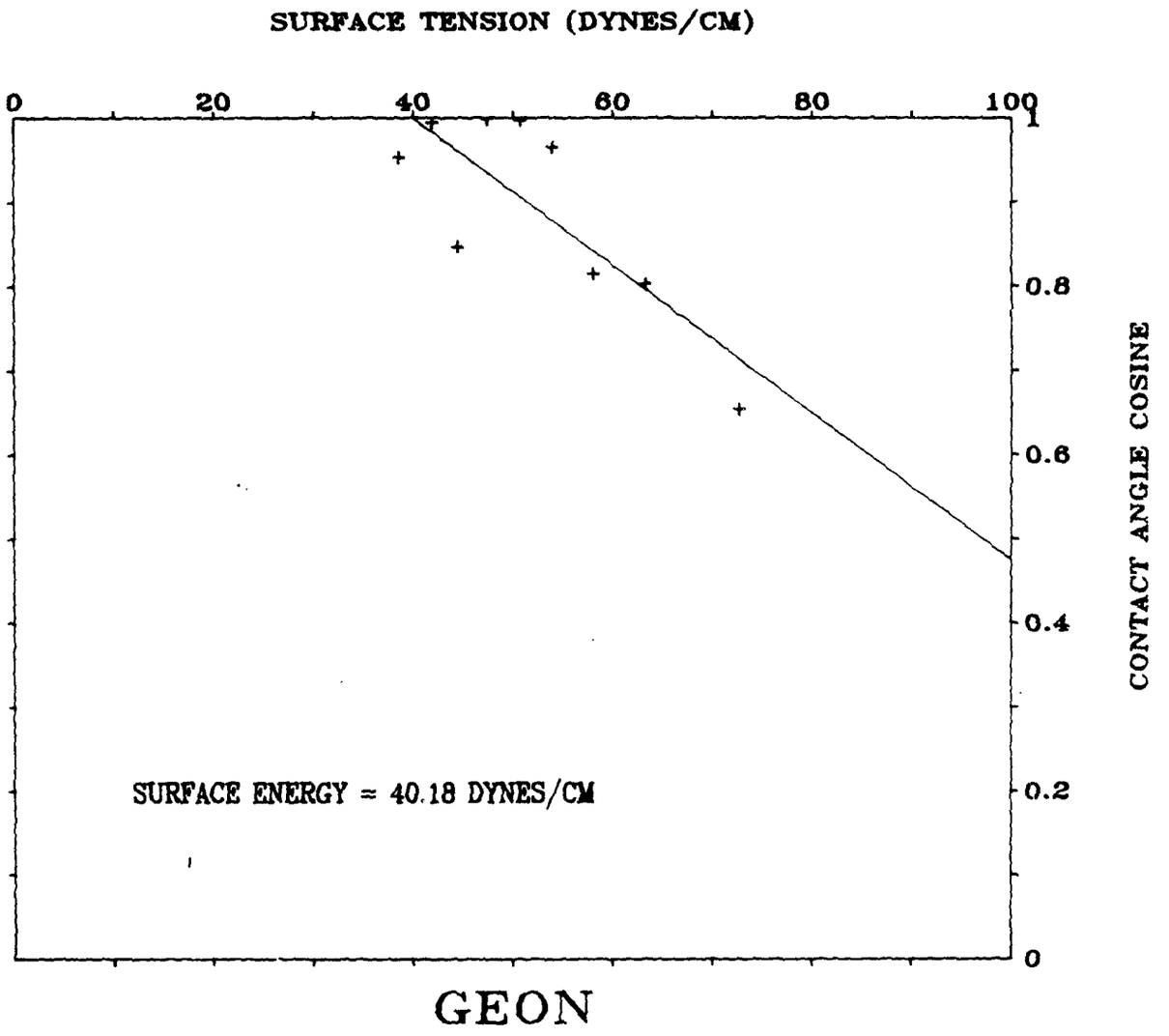

SURFACE ENERGY (DYNES/CM)
0
20
40
60
80
100
0.8
0.6
0.4
0.2
0
CONTACT ANGLE COSINE
SURFACE ENERGY = 28.90 DYNES/CM
POLYPROPYLENE

- FIGURE (72) -

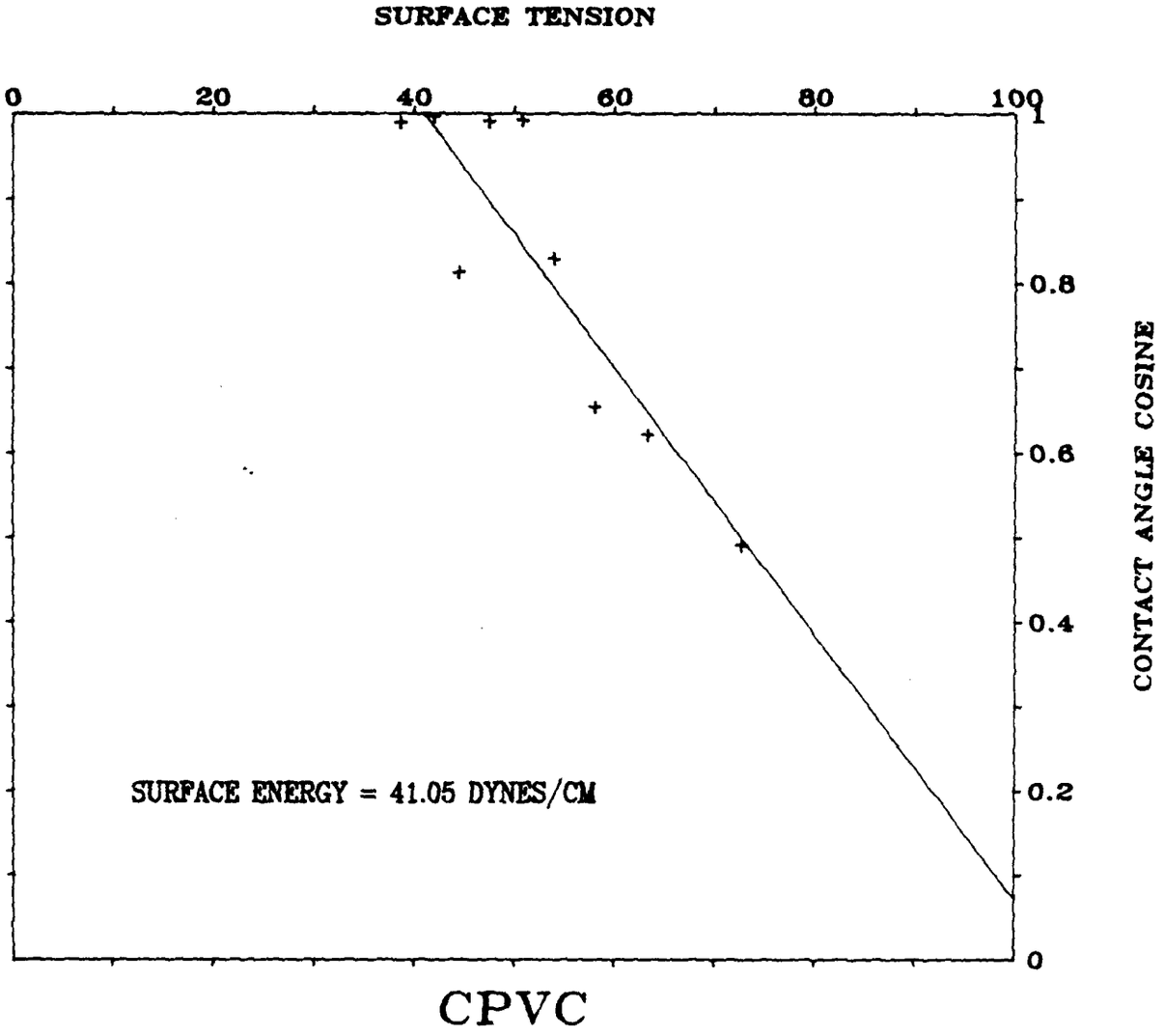


HYCAR (REC. #4)

- FIGURE (73) -


SURFACE TENSION (DYNES/CM)
0
20
40
60
80
100
1
0.8
0.6
0.4
0.2
0
CONTACT ANGLE COSINE
SURFACE ENERGY = 40.18 DYNES/CM
GEON

- FIGURE (74) -



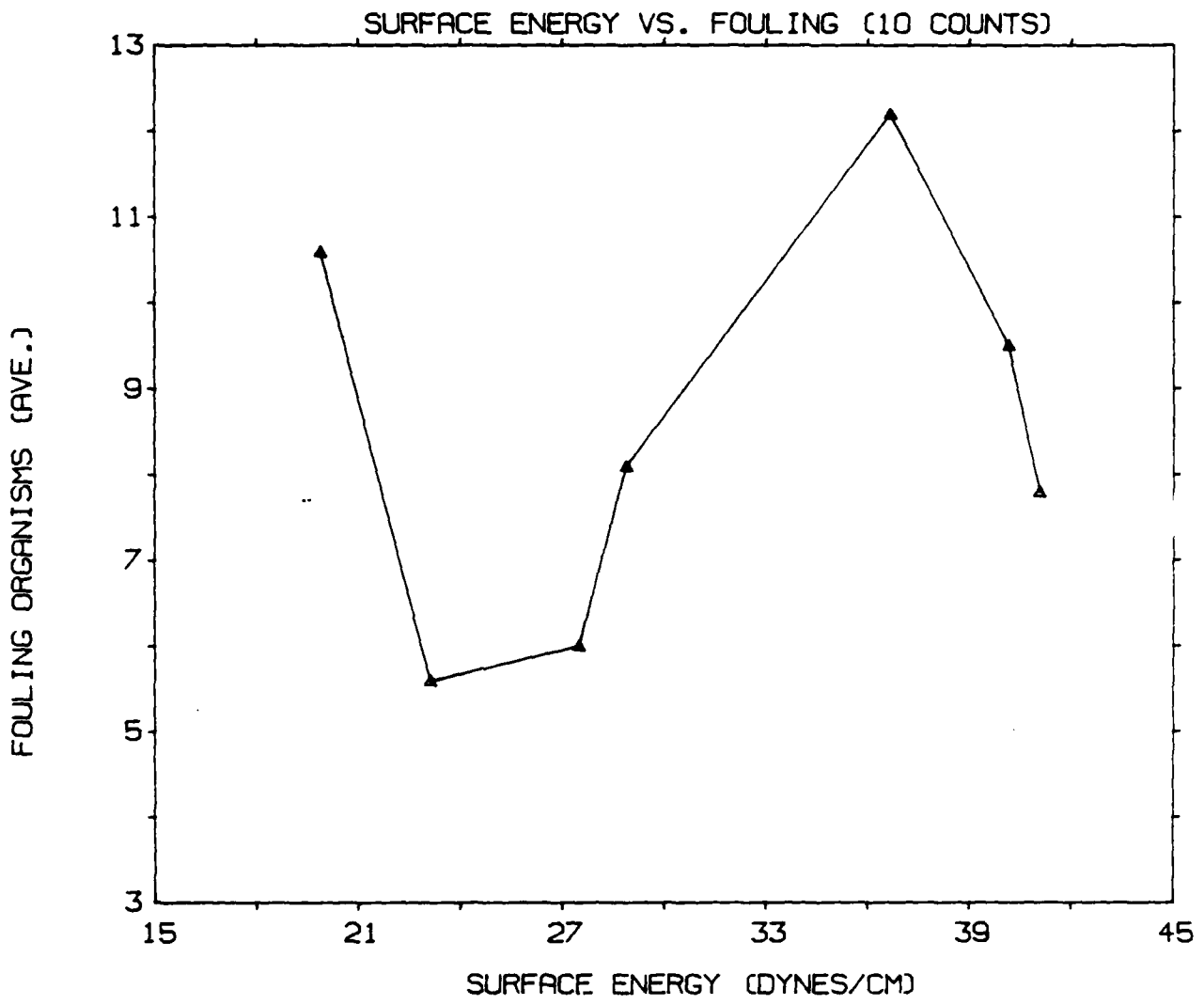
CPVC

APPENDIX E - EXPERIMENT TWO:

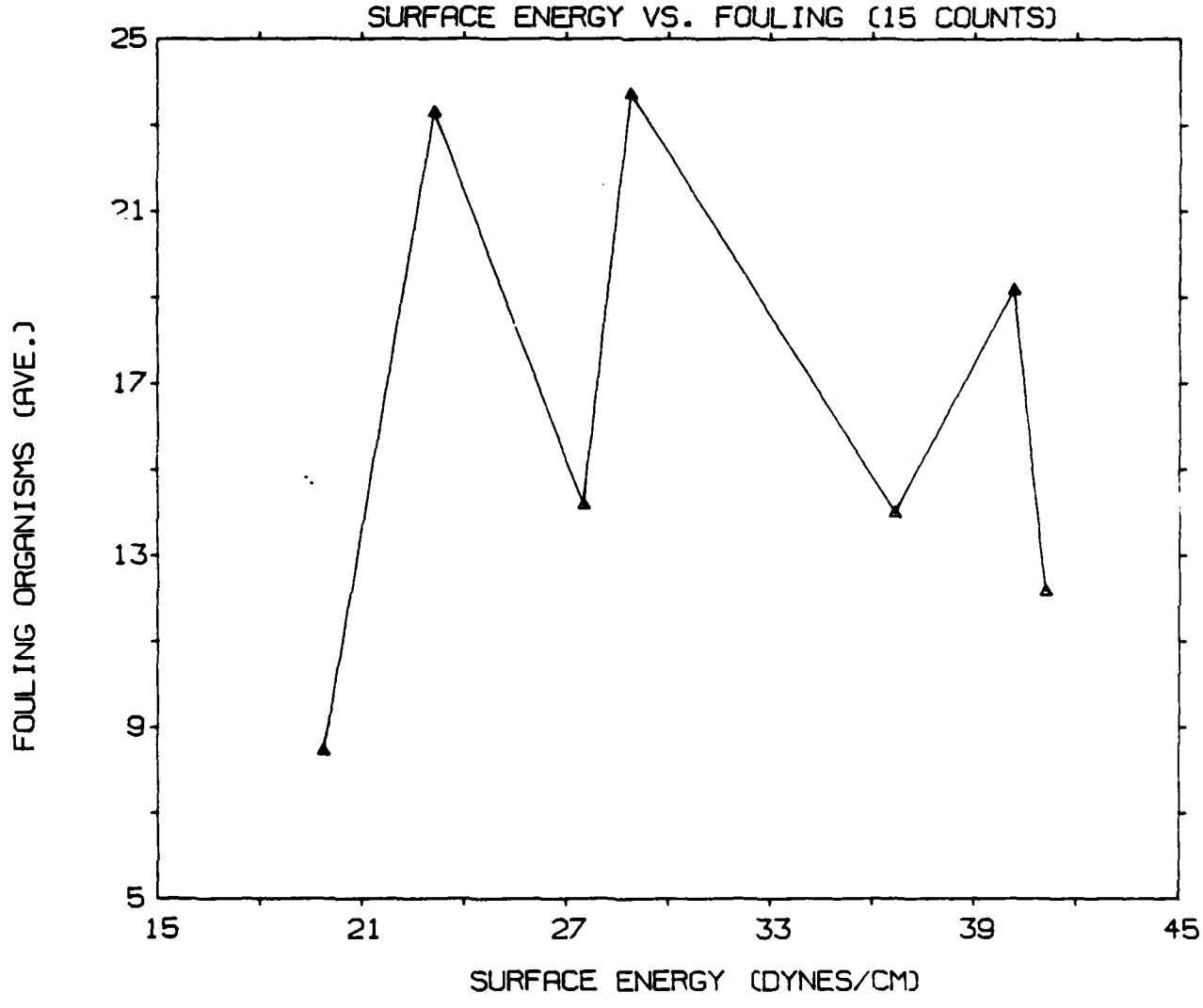
GRAPHS OF SURFACE ENERGY VS. FOULING

AS DETERMINED BY HBS/DISSECTION MICROSCOPE ANALYSIS

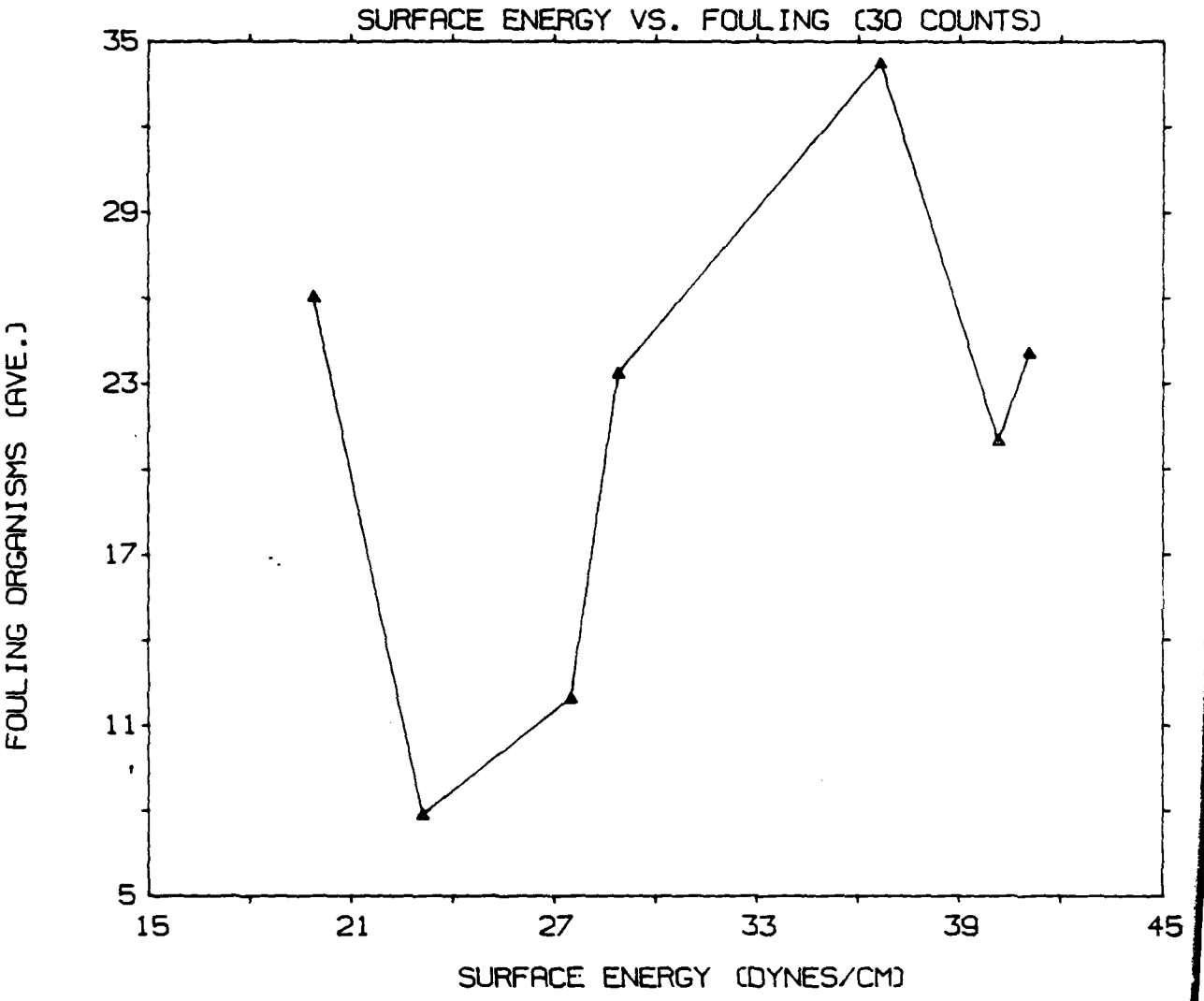
- FIGURE (75) -


SURFACE ENERGY VS. FOULING (10 COUNTS)
FOULING ORGANISMS (AVE.)
13
11
9
7
5
3
15
21
27
33
39
45
SURFACE ENERGY (DYNES/CM)

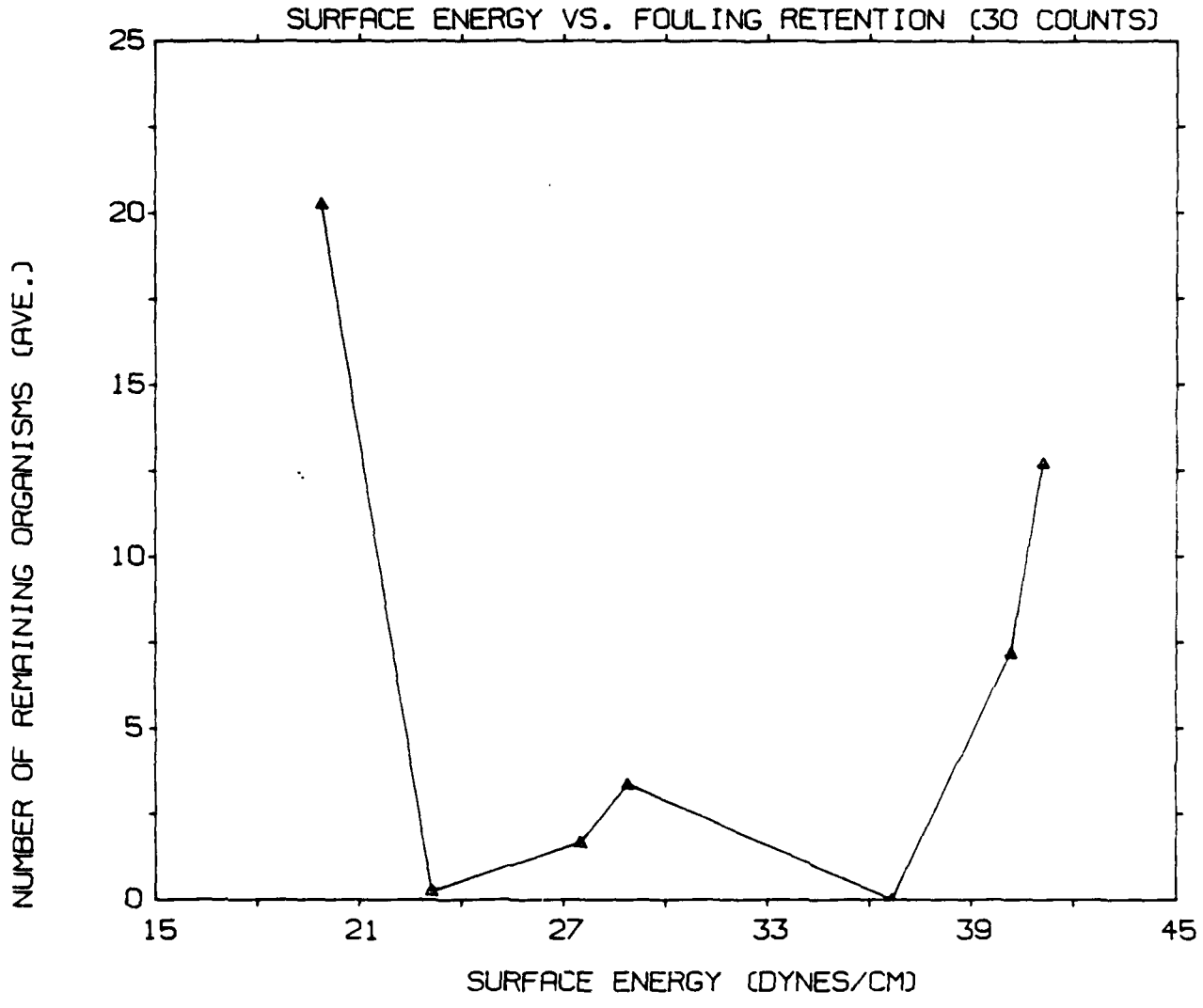
- FIGURE (76) -


SURFACE ENERGY VS. FOULING (15 COUNTS)
FOULING ORGANISMS (AVE.)
25
21
17
13
9
5
15
21
27
33
39
45
SURFACE ENERGY (DYNES/CM)

- FIGURE (77) -


SURFACE ENERGY VS. FOULING (30 COUNTS)
FOULING ORGANISMS (AVE.)
35
29
23
17
11
5
15
21
27
33
39
45
SURFACE ENERGY (DYNES/CM)

- FIGURE (78) -


SURFACE ENERGY VS. FOULING RETENTION (30 COUNTS)
NUMBER OF REMAINING ORGANISMS (AVE.)
25
20
15
10
5
0
15
21
27
33
39
45
SURFACE ENERGY (DYNES/CM)

APPENDIX F - EXPERIMENT TWO:

PHOTOGRAPHS OF HBS/SEM ANALYSIS OF SUBSTRATES

- PLATE (1) -

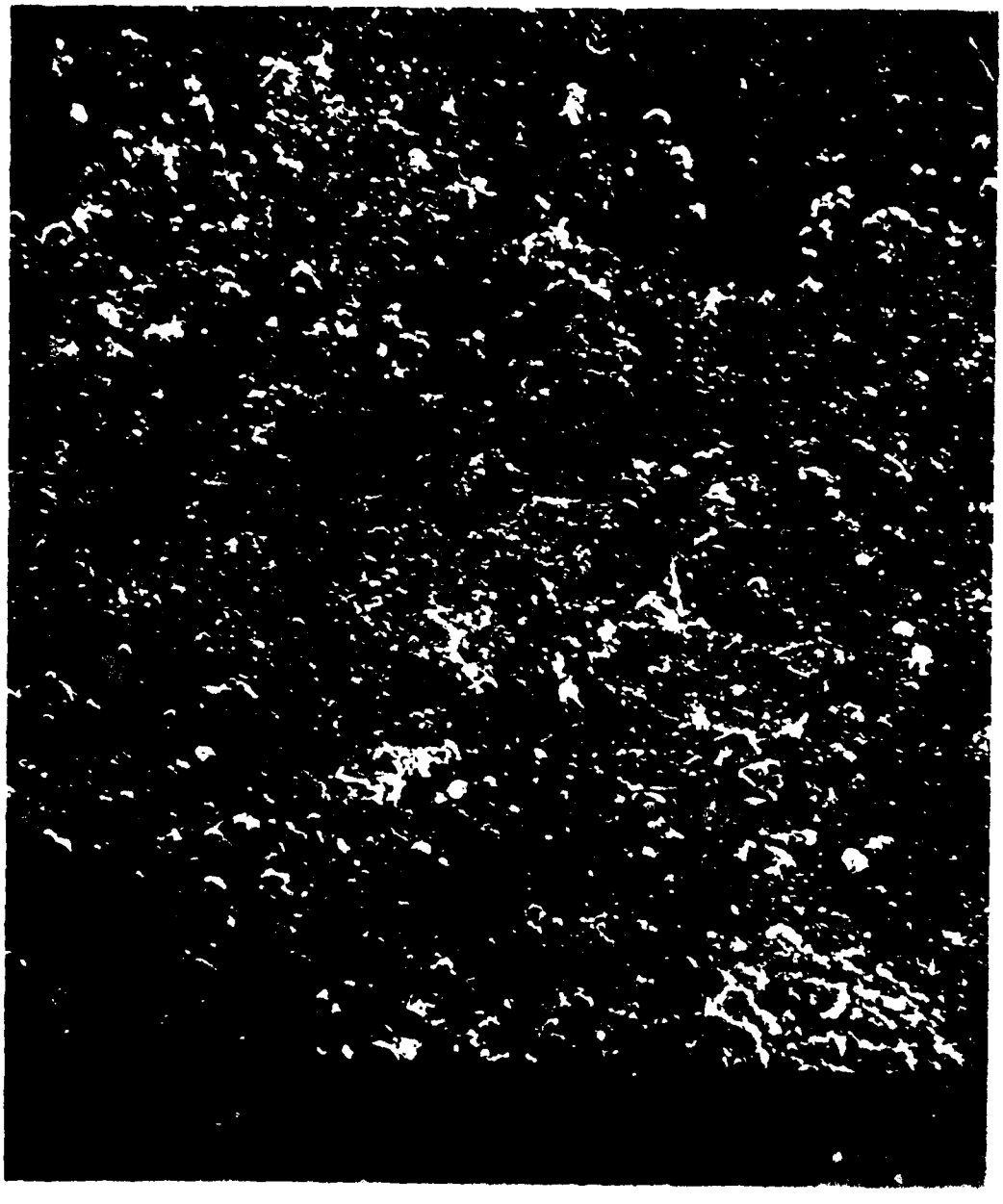
TEFLON BEFORE HBS EXPOSURE (100 X)

- PLATE (2) -

TEFLON AFTER HBS EXPOSURE (100 X)

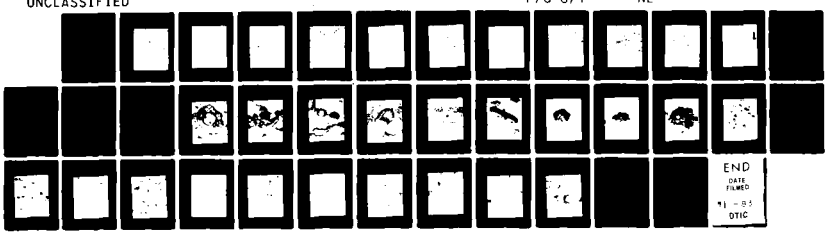
- PLATE (3) -

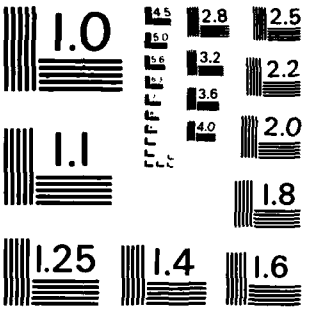
DIMETHYL POLYSILOXANE BEFORE HBS EXPOSURE (100 X)



AD-A134 290 SURFACE ENERGIES AND CHEMICAL ANALYSIS OF THE INITIAL STAGES OF MARINE MICROBIOLOGICAL FOULING(U) NAVAL ACADEMY ANNAPOLIS MD M J OLSON 20 JUN 83 USNA-TSPR-127 3/3

UNCLASSIFIED F/G 8/1 NL

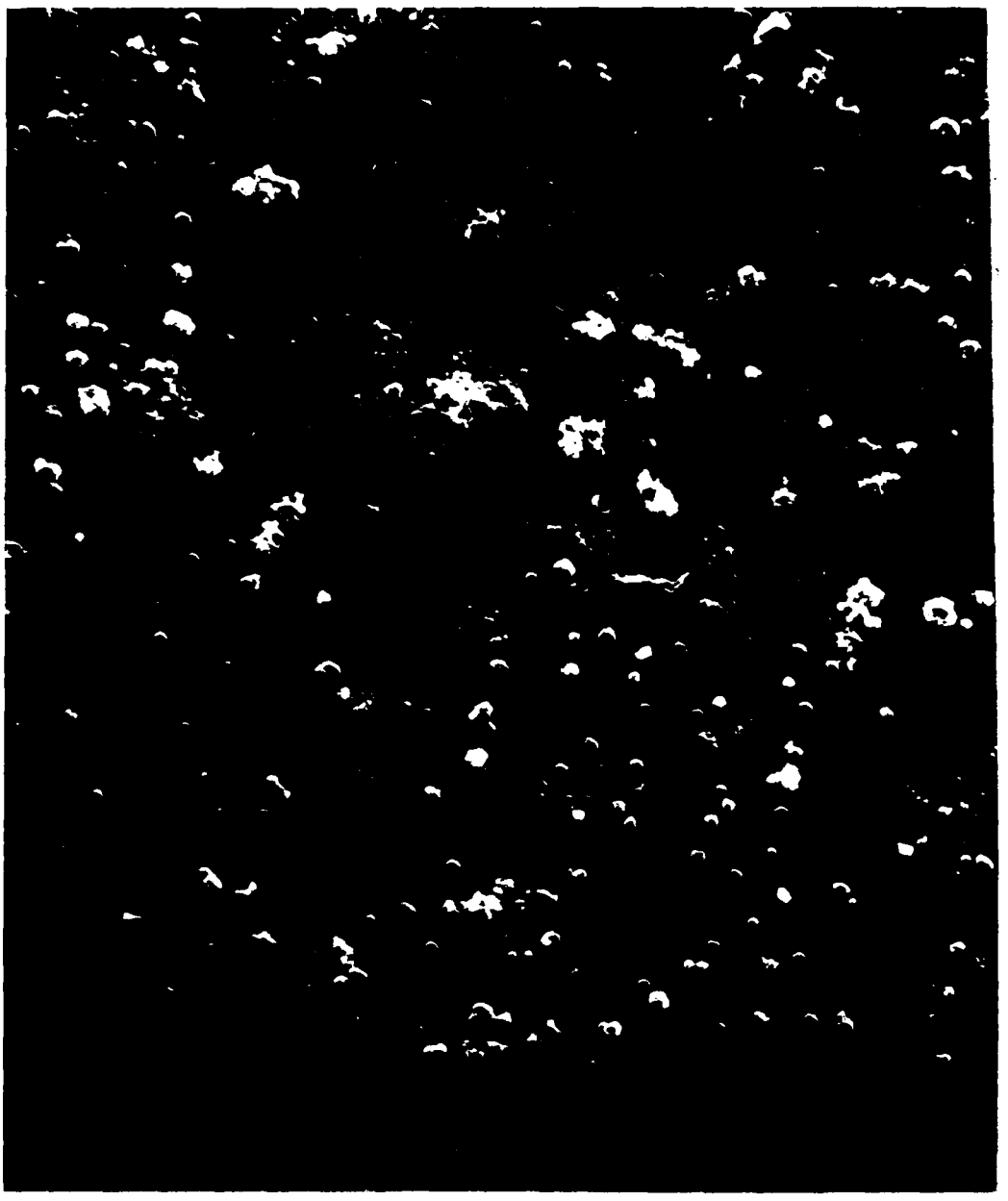




MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS - 1963 - A

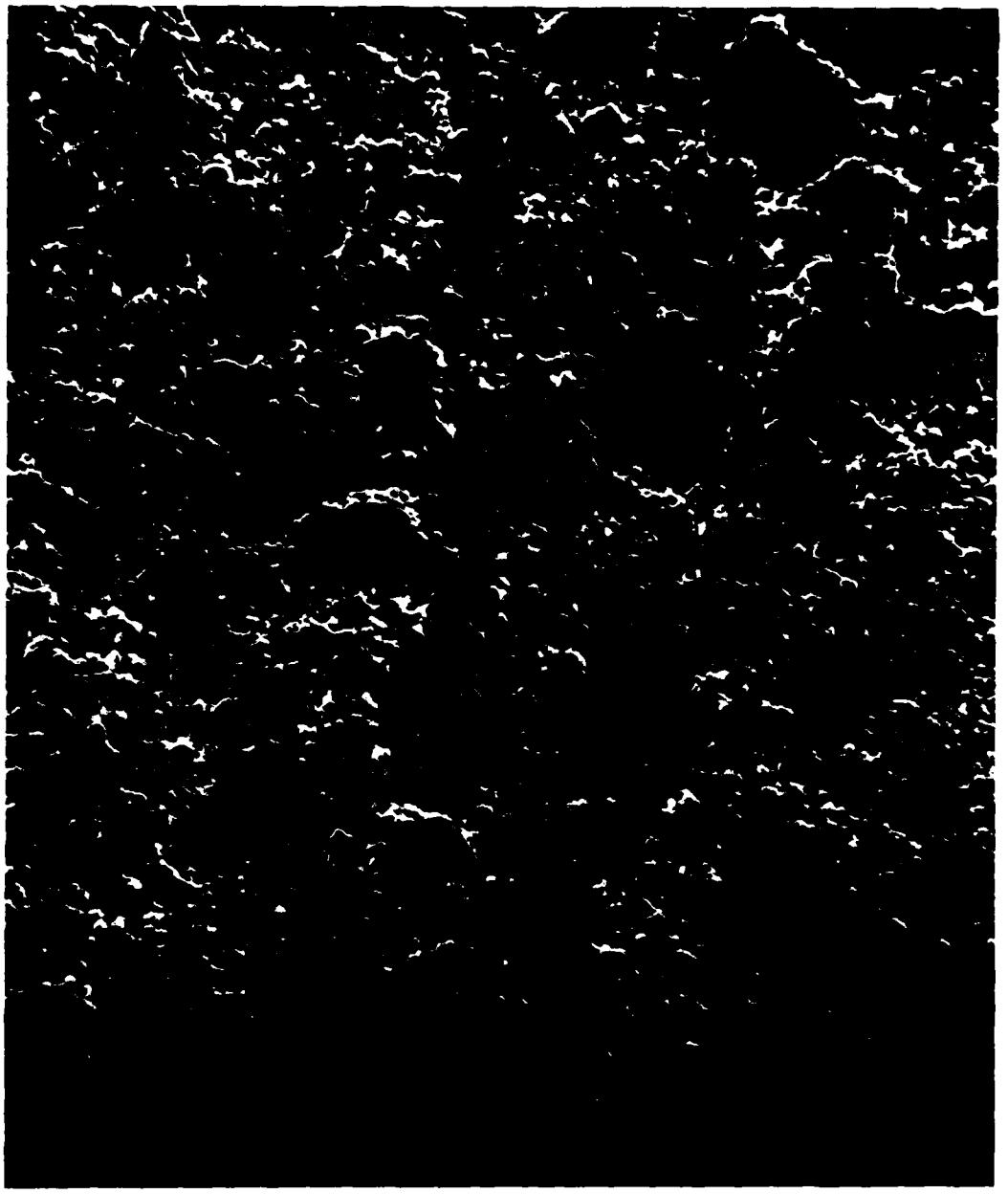
- PLATE (4) -

DIMETHYL POLYSILOXANE AFTER HBS EXPOSURE (100 X)



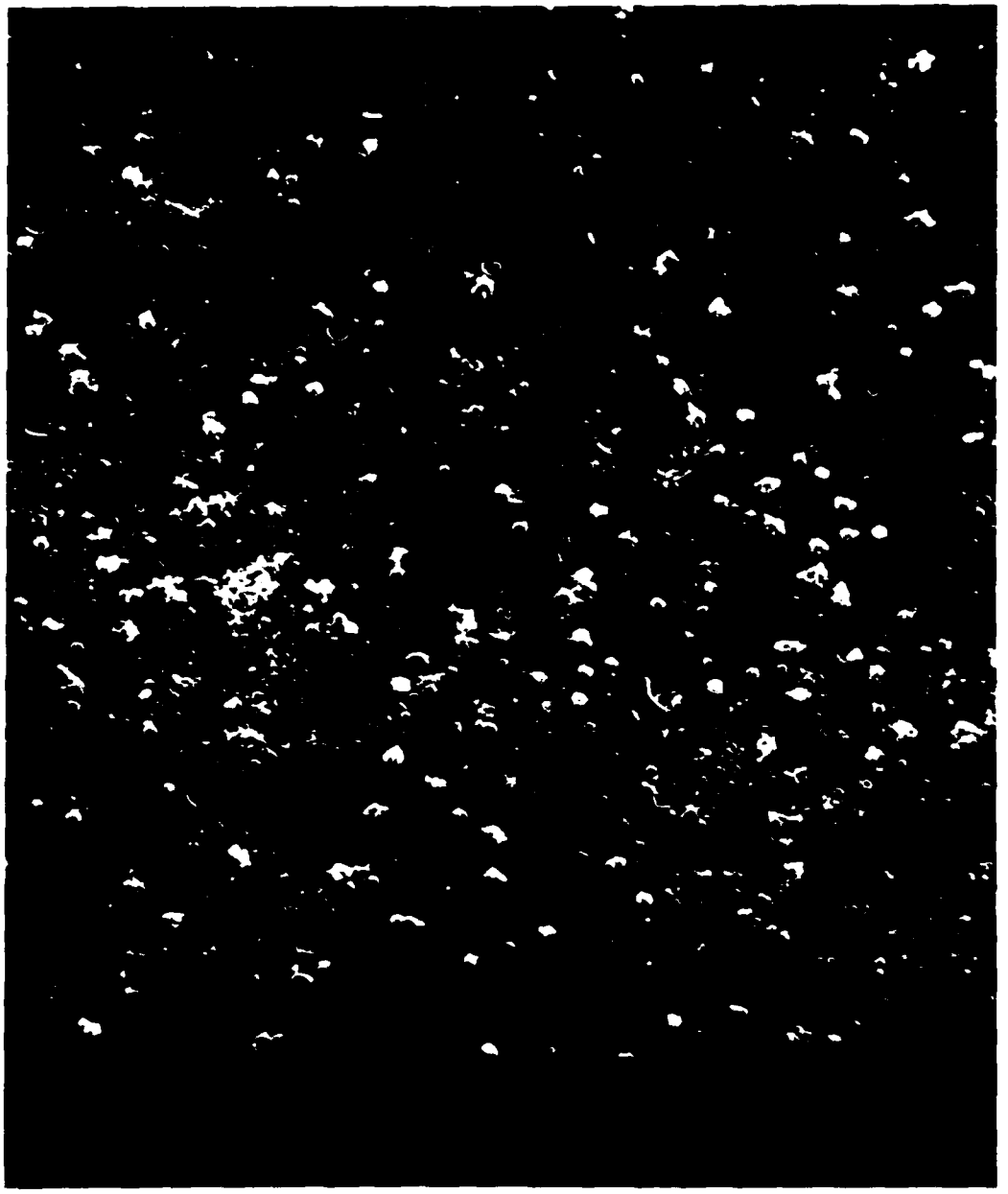
- PLATE (5) -

SILASTIC BEFORE HBS EXPOSURE (100 X)



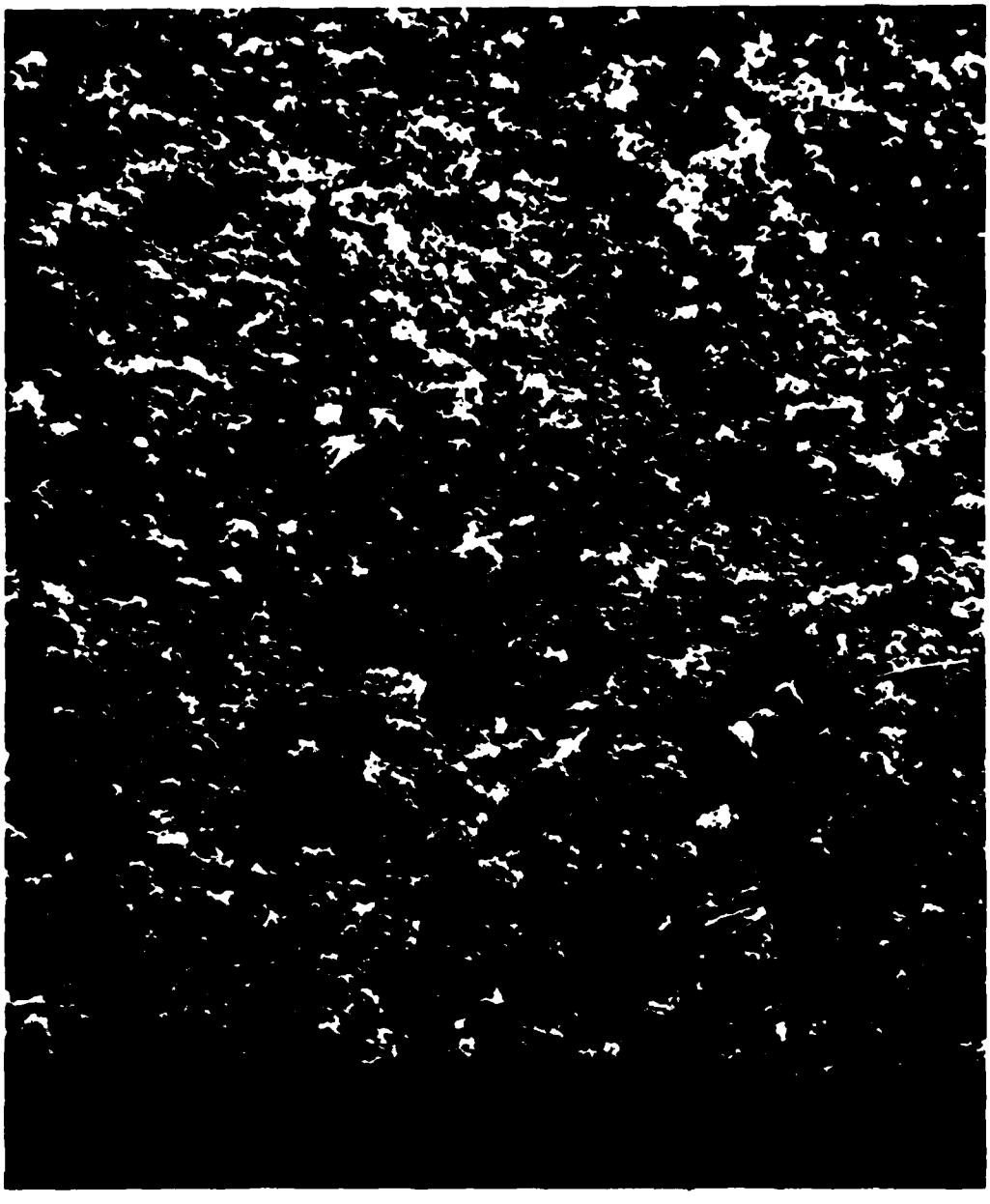
- PLATE (6) -

SILASTIC AFTER HBS EXPOSURE (100 X)



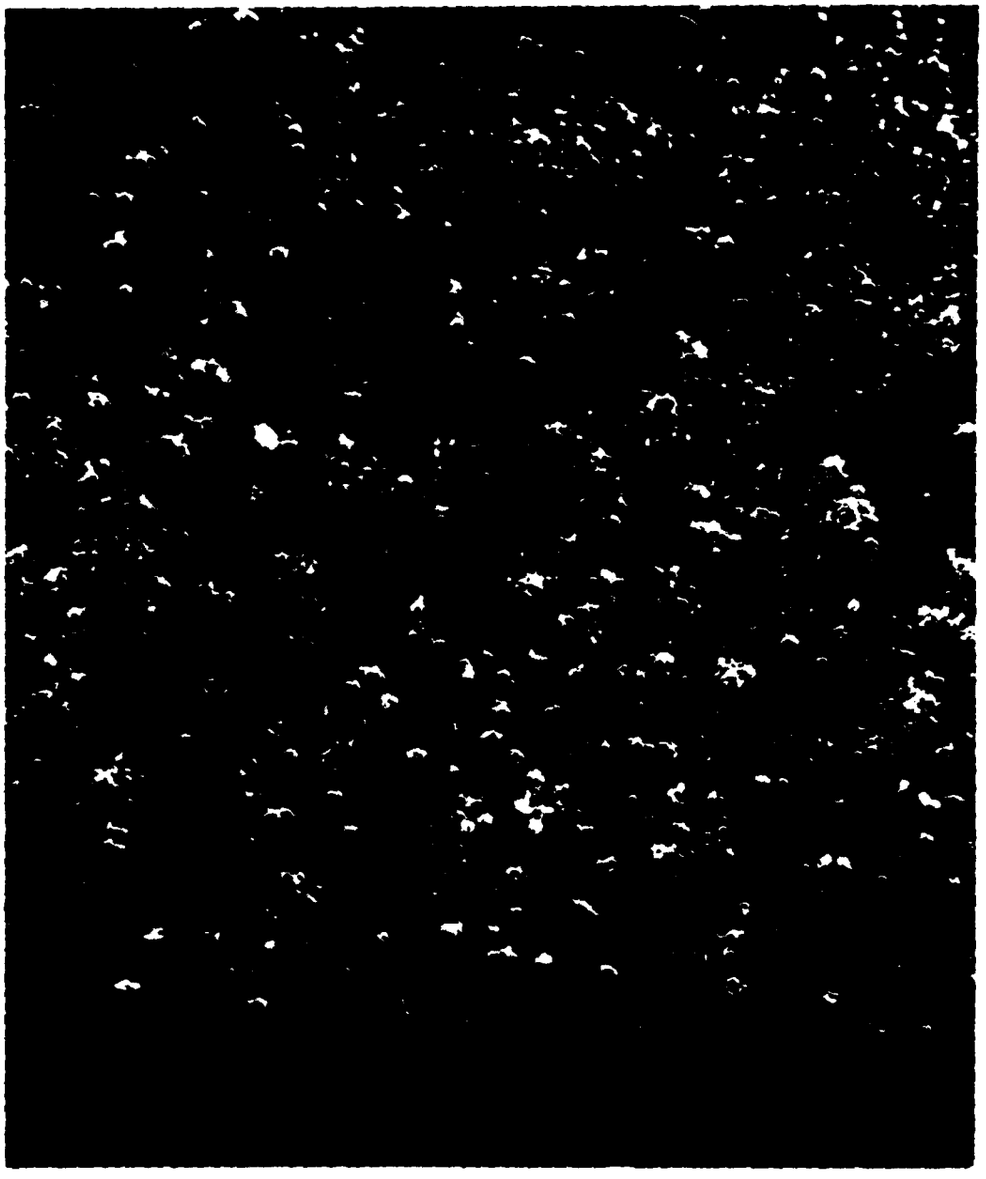
- PLATE (7) -

POLYPROPYLENE BEFORE HBS EXPOSURE (100 X)



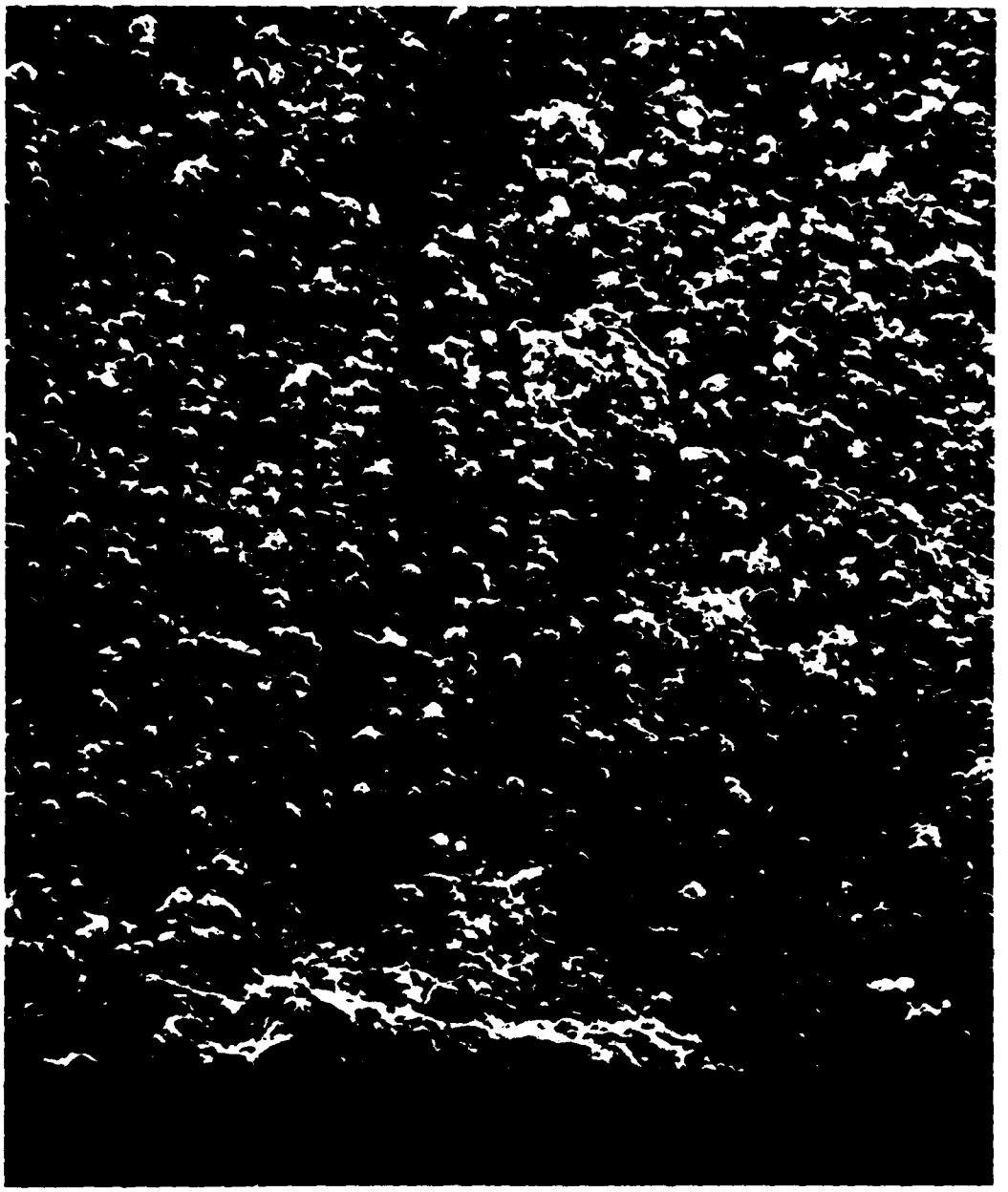
- PLATE (8) -

POLYPROPYLENE AFTER HBS EXPOSURE (100 X)



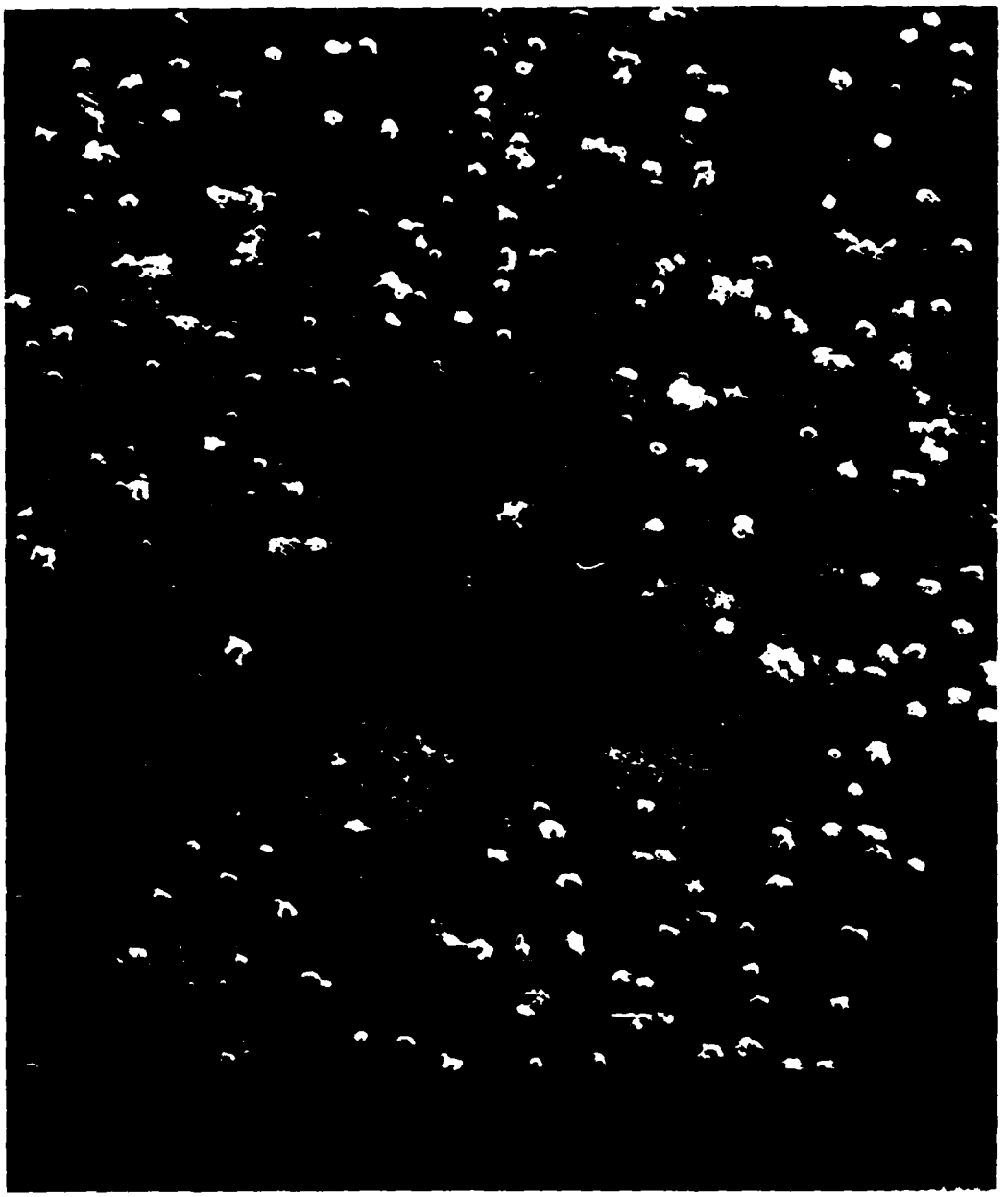
- PLATE (9) -

HYCAR BEFORE HBS EXPOSURE (100 X)



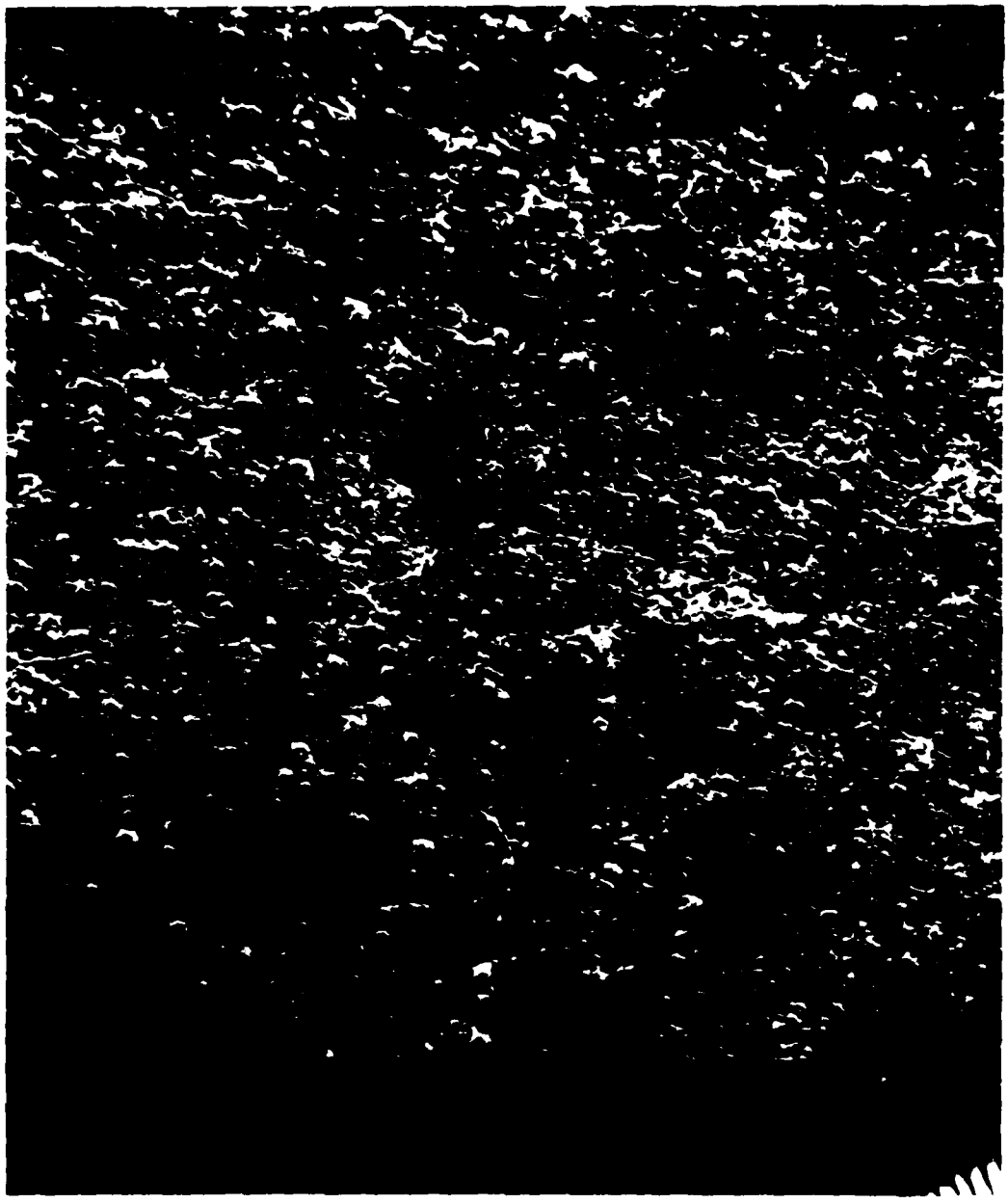
- PLATE (10) -

HYCAR AFTER HBS EXPOSURE (100 X)



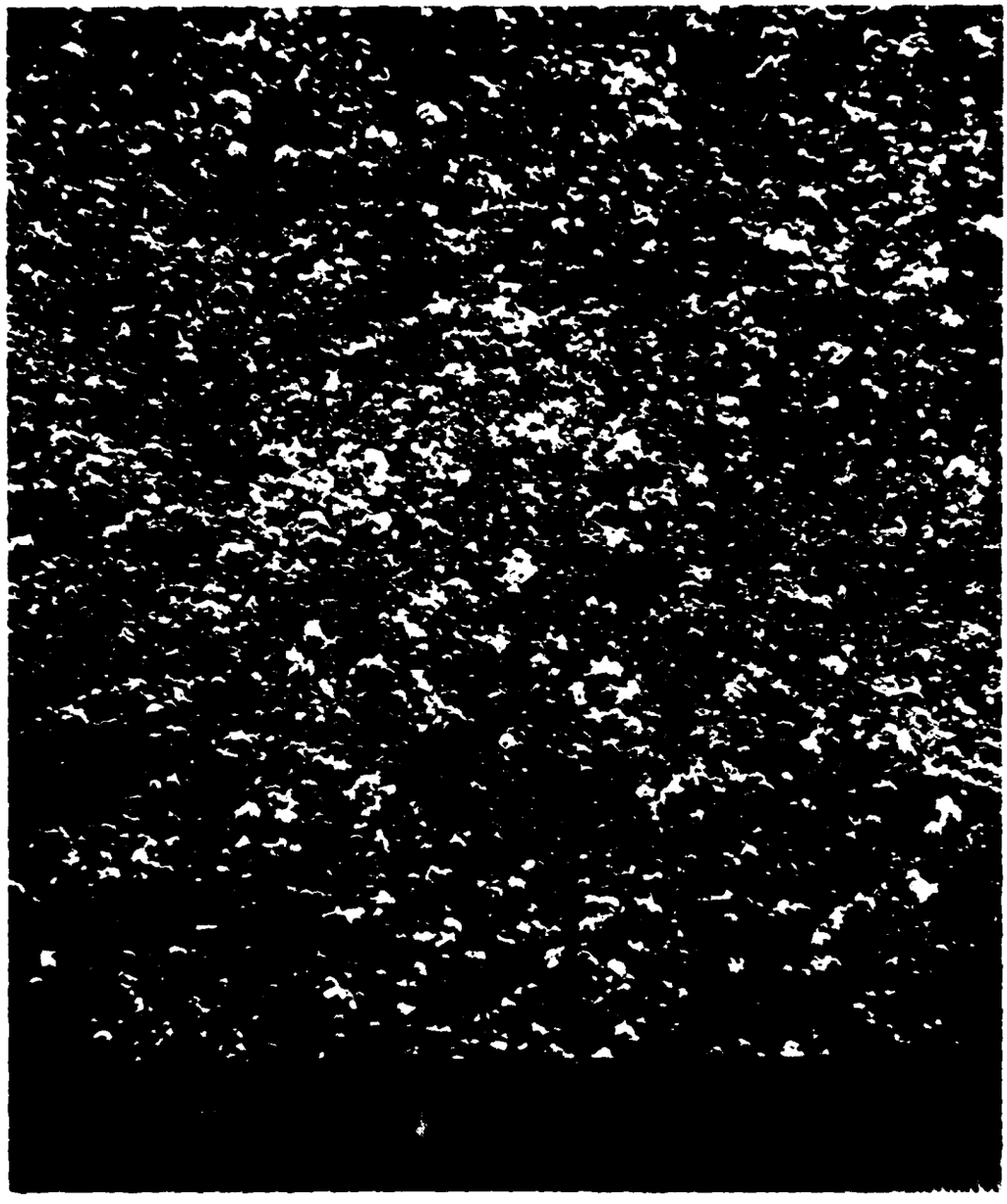
- PLATE (11) -

GEON BEFORE HBS EXPOSURE (100 X)



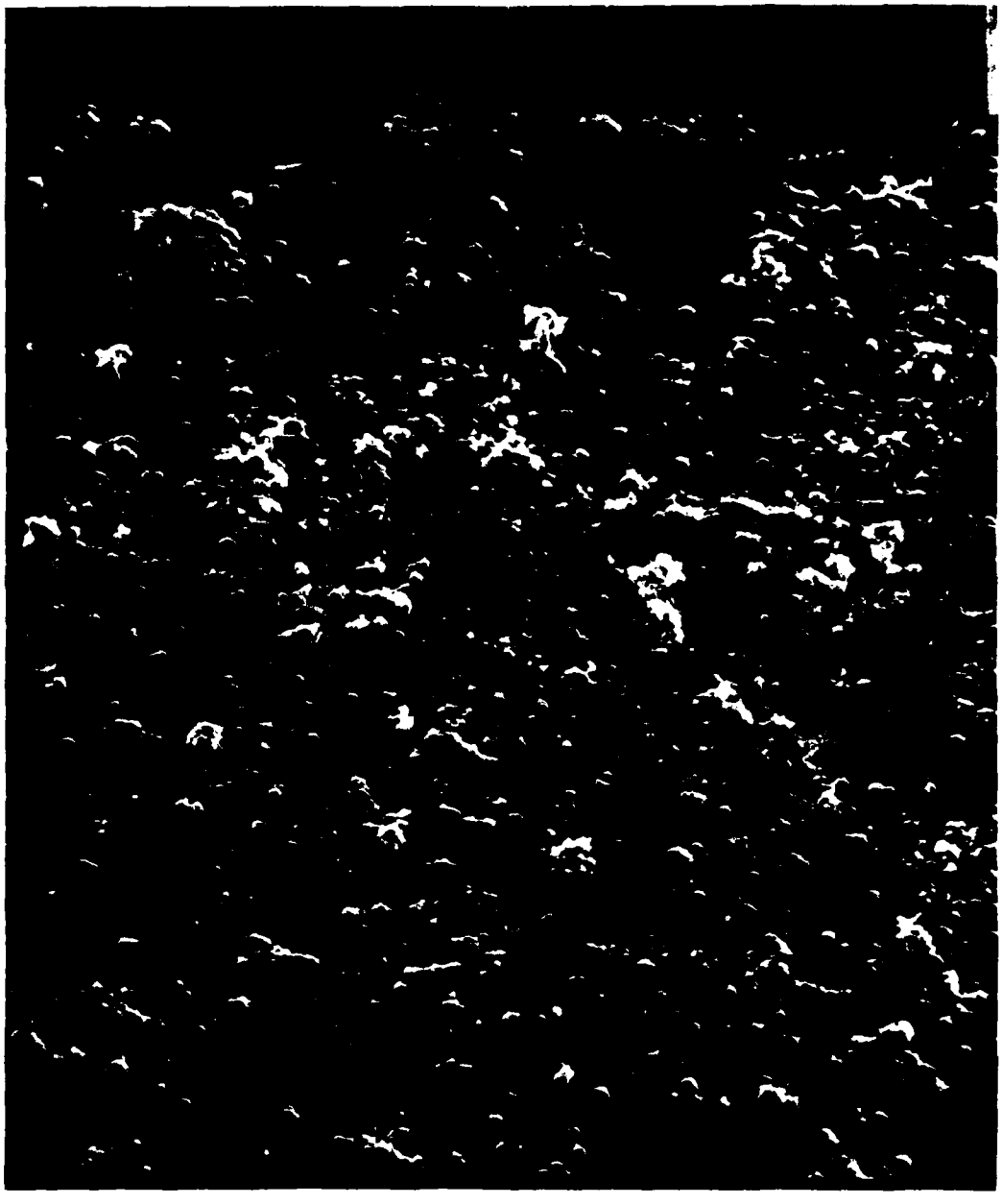
- PLATE (12) -

GEON AFTER HBS EXPOSURE (100 X)



- PLATE (13) -

CPVC BEFORE HBS EXPOSURE (100 X)



- PLATE (14) -

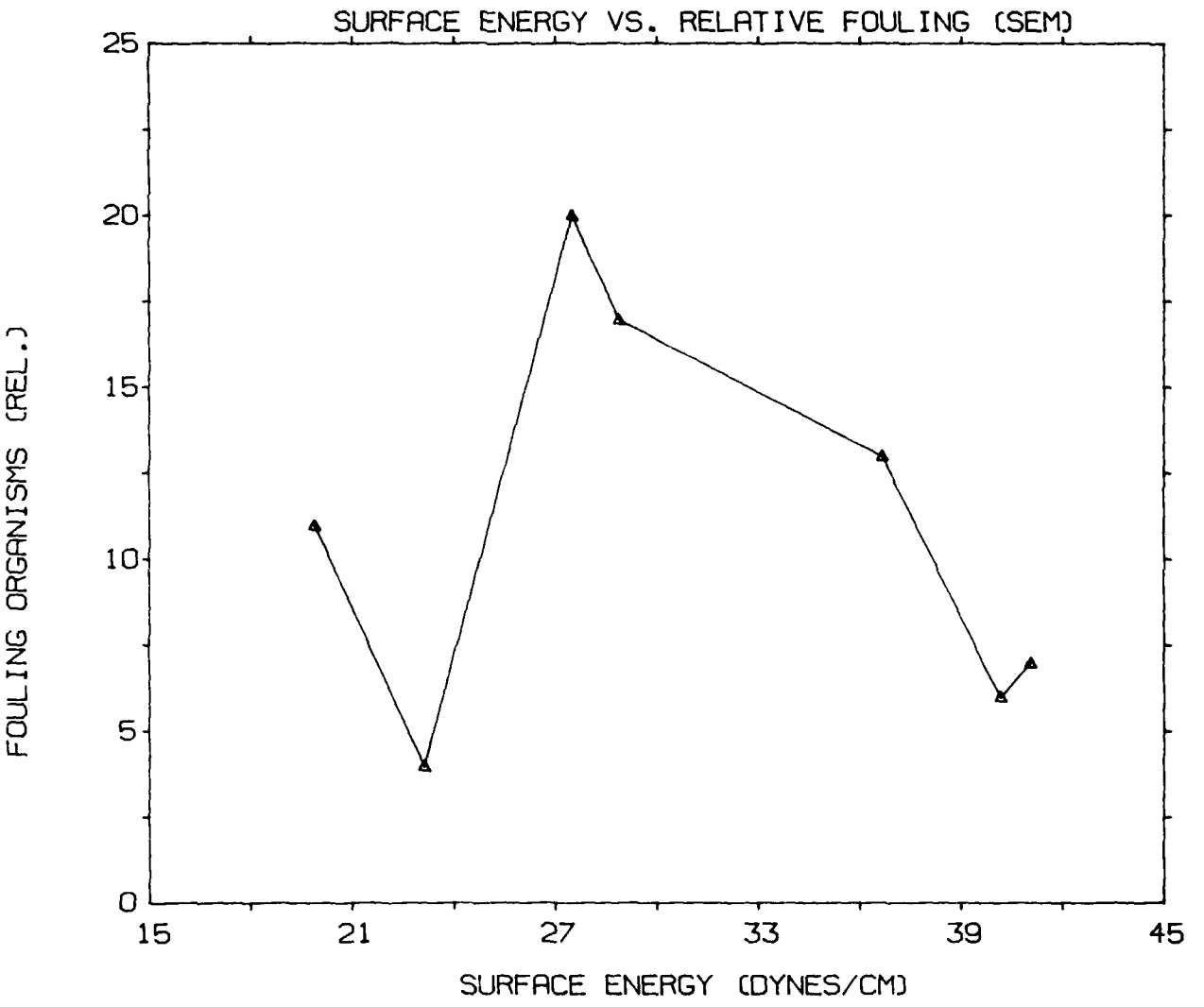
CPVC AFTER HBS EXPOSURE (100 X)

APPENDIX G - EXPERIMENT TWO:

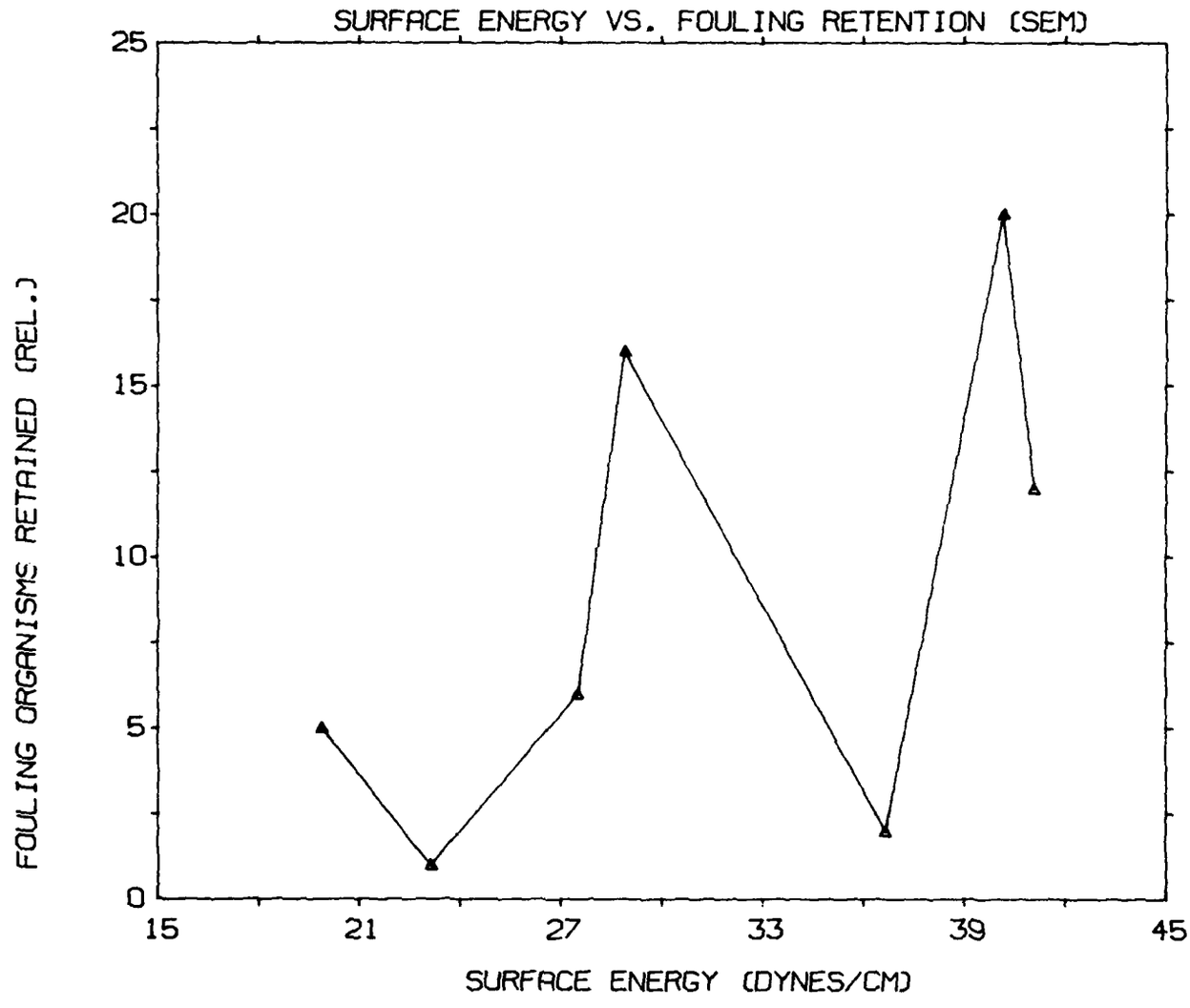
GRAPHS OF SURFACE ENERGY VS. RELATIVE FOULING

AS DETERMINED BY HBS/SEM ANALYSIS

- FIGURE (79) -


SURFACE ENERGY VS. RELATIVE FOULING (SEM)
FOULING ORGANISMS (REL.)
25
20
15
10
5
0
15
21
27
33
39
45
SURFACE ENERGY (DYNES/CM)

- FIGURE (80) -

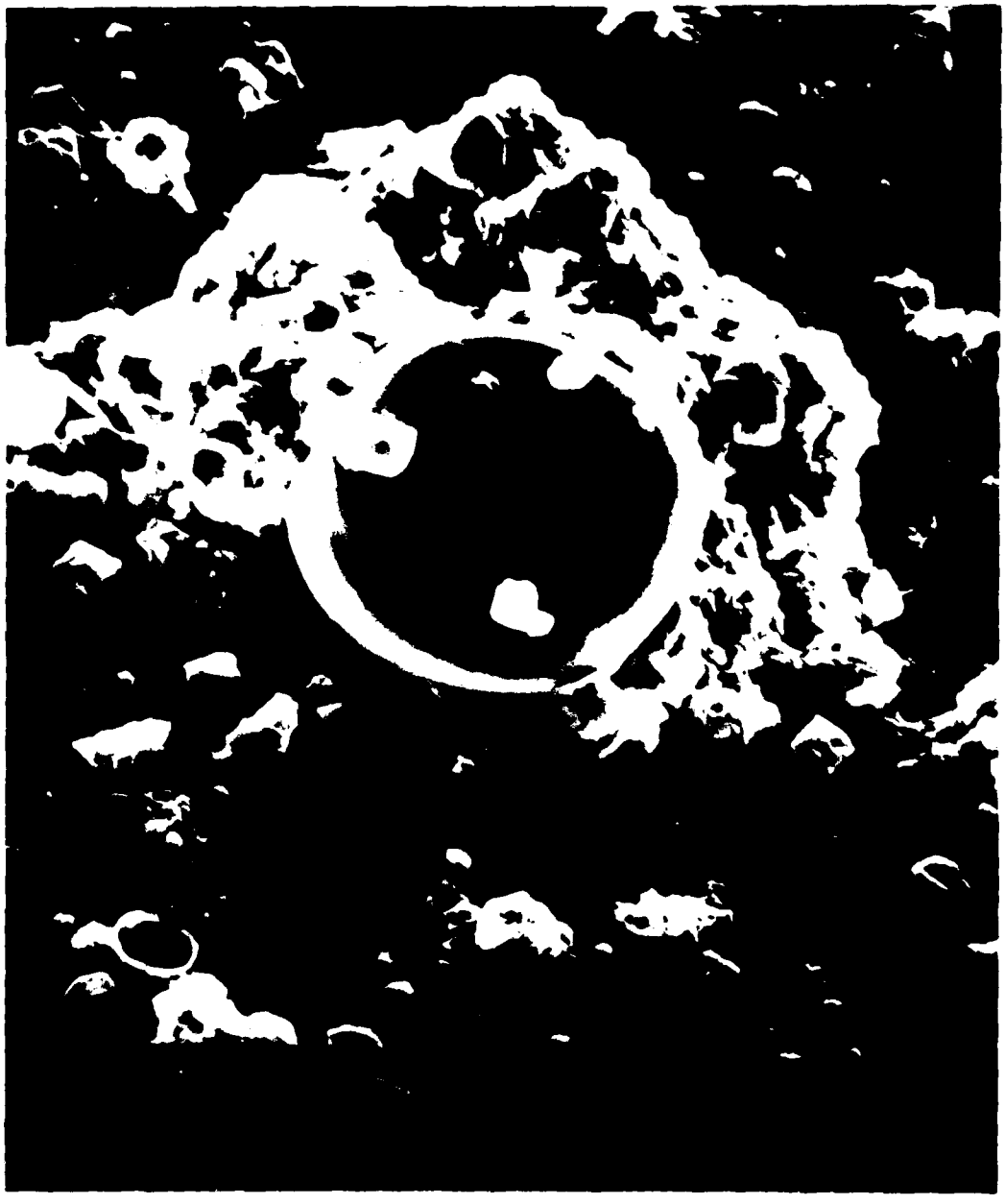

SURFACE ENERGY VS. FOULING RETENTION (SEM)
FOULING ORGANISMS RETAINED (REL.)
25
20
15
10
5
0
15
21
27
33
39
45
SURFACE ENERGY (DYNES/CM)

APPENDIX H - EXPERIMENT TWO:

PHOTOGRAPHS OF SOME PRIMARY FOULING ORGANISMS

- PLATE (15) -

CENTRIC DIATOM NYDROID TUBULARIA OR
NYDROID CROCEA (Sieburth, 1975) (600 X)

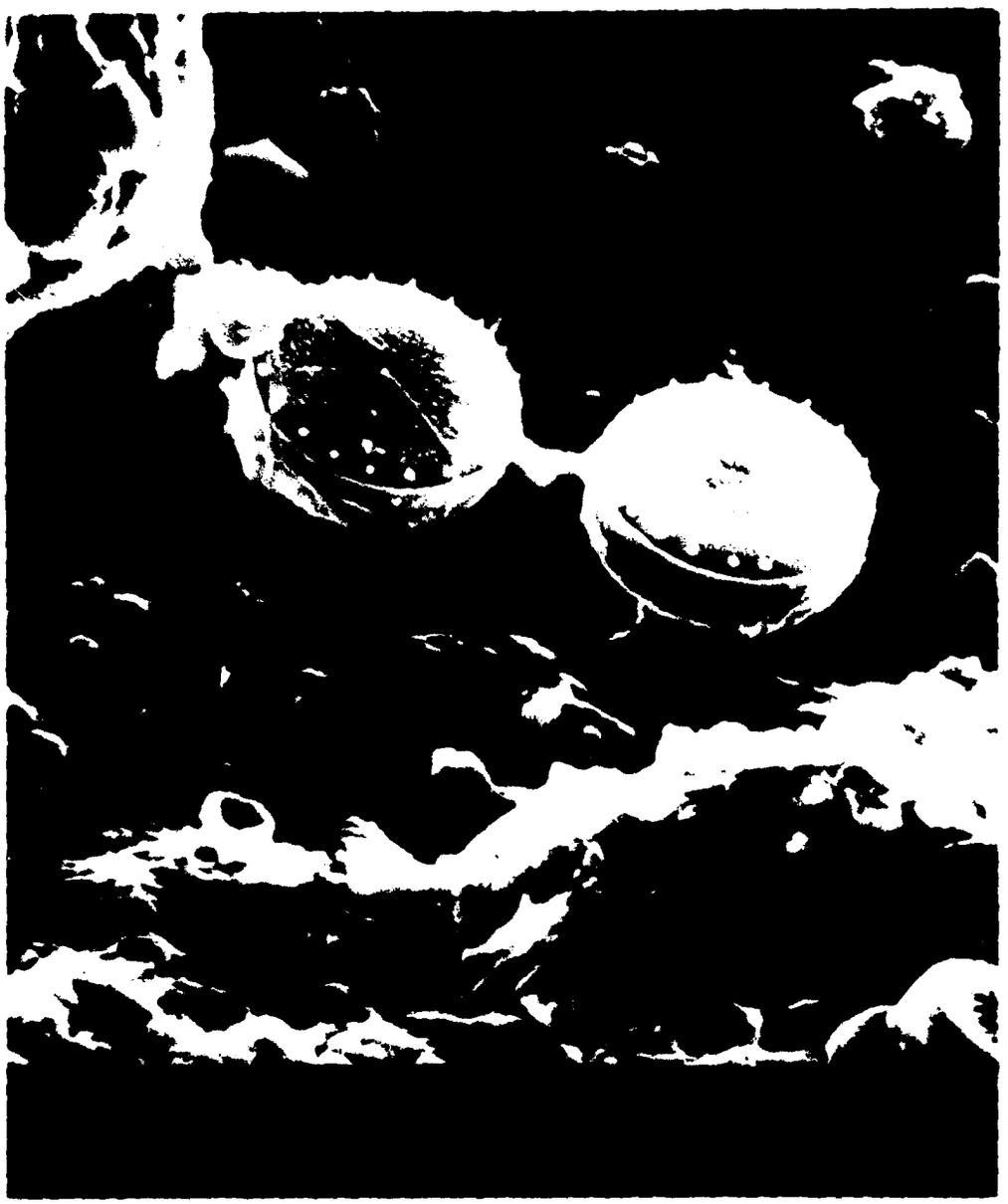


- PLATE (16) -

CENTRIC DIATOM THALASSIOSIRA NORDENSKJOLDII
(Sieburth, 1975) (1100 X)

- PLATE (17) -

CENTRIC DIATOM THALASSIOSIRA (Sieburth, 1975) (1100 X)



- PLATE (18) -

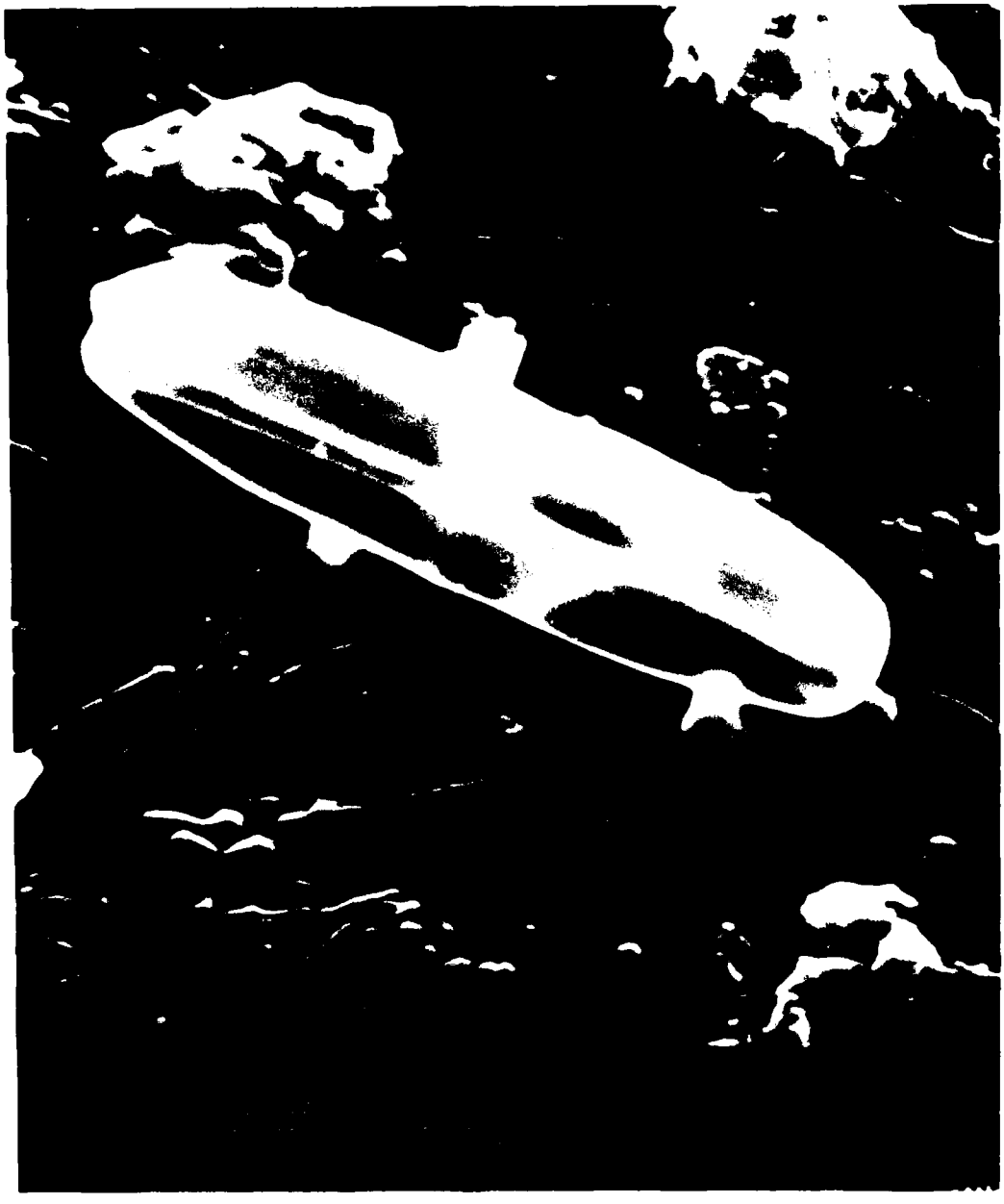
UNIDENTIFIED ORGANISM,
POSSIBLY A FORM OF PENNATE DIATOM (3000 X)

- PLATE (19) -

SALT CRYSTALS FORMING GEOMETRIC PATTERN (3000 X)

- PLATE (20) -

PROTOZOAN, POSSIBLY A TYPE OF EUGLENA (Jahn, 1949) (1700 X)

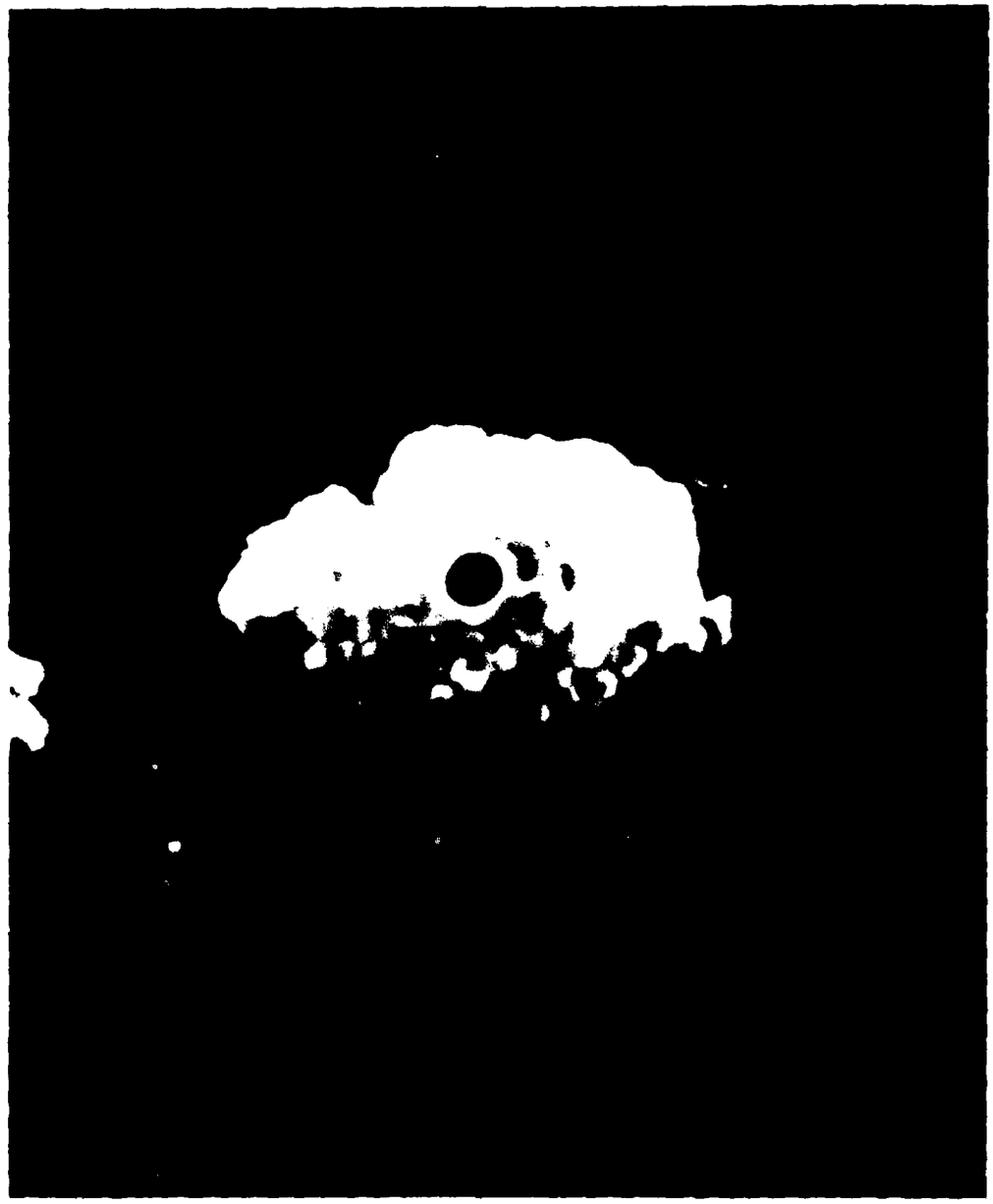


- PLATE (21) -

UNIDENTIFIED ORGANISM - "SHEEP" (2000 X)

- PLATE (22) -

UNIDENTIFIED ORGANISM - "SHEEP" (2000 X)

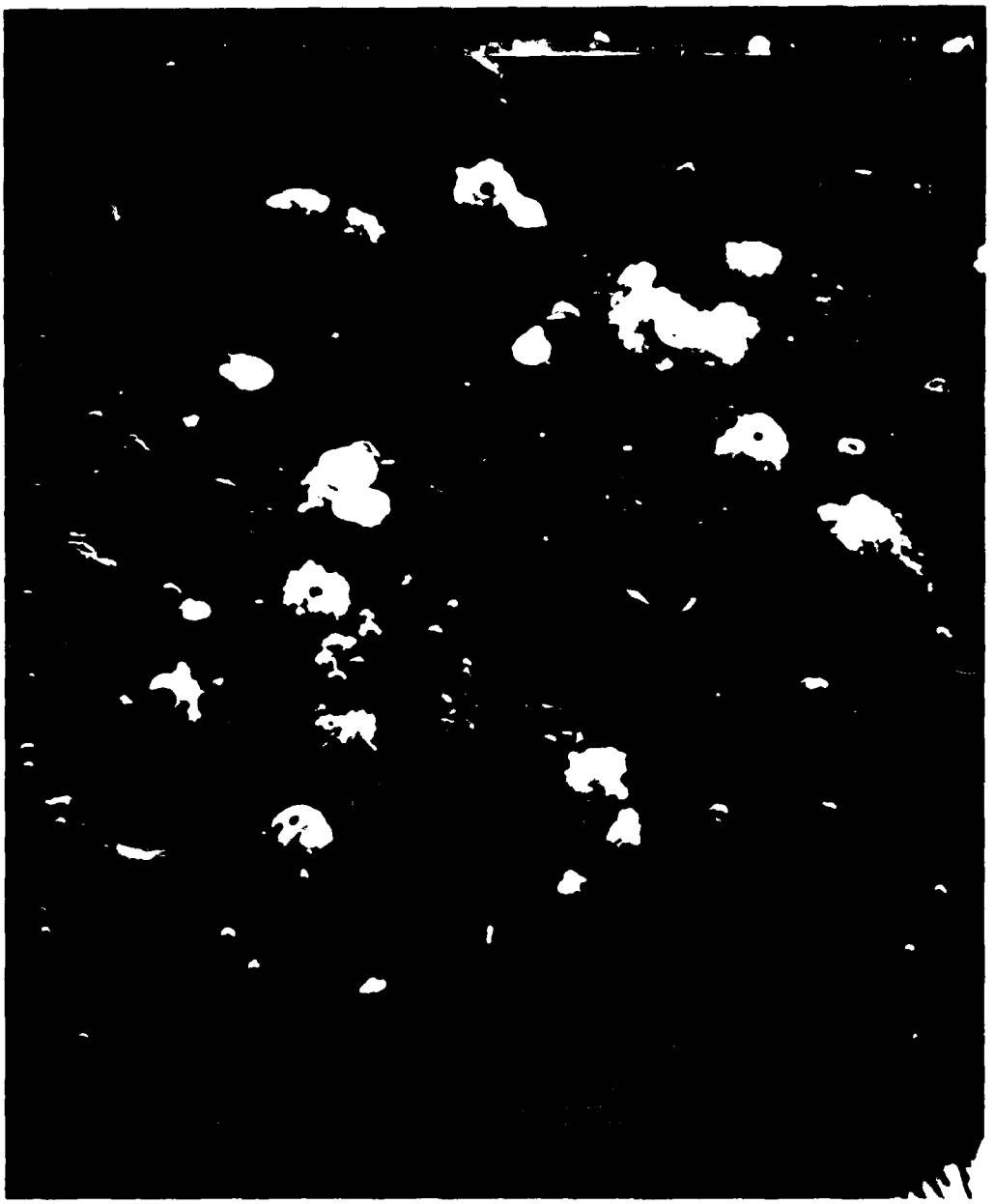


- PLATE (23) -

UNIDENTIFIED ORGANISM - "SHEEP" (4000 X)

- PLATE (24) -

COLONY OF "SHEEP" (400 X)



APPENDIX I - EXPERIMENT THREE:

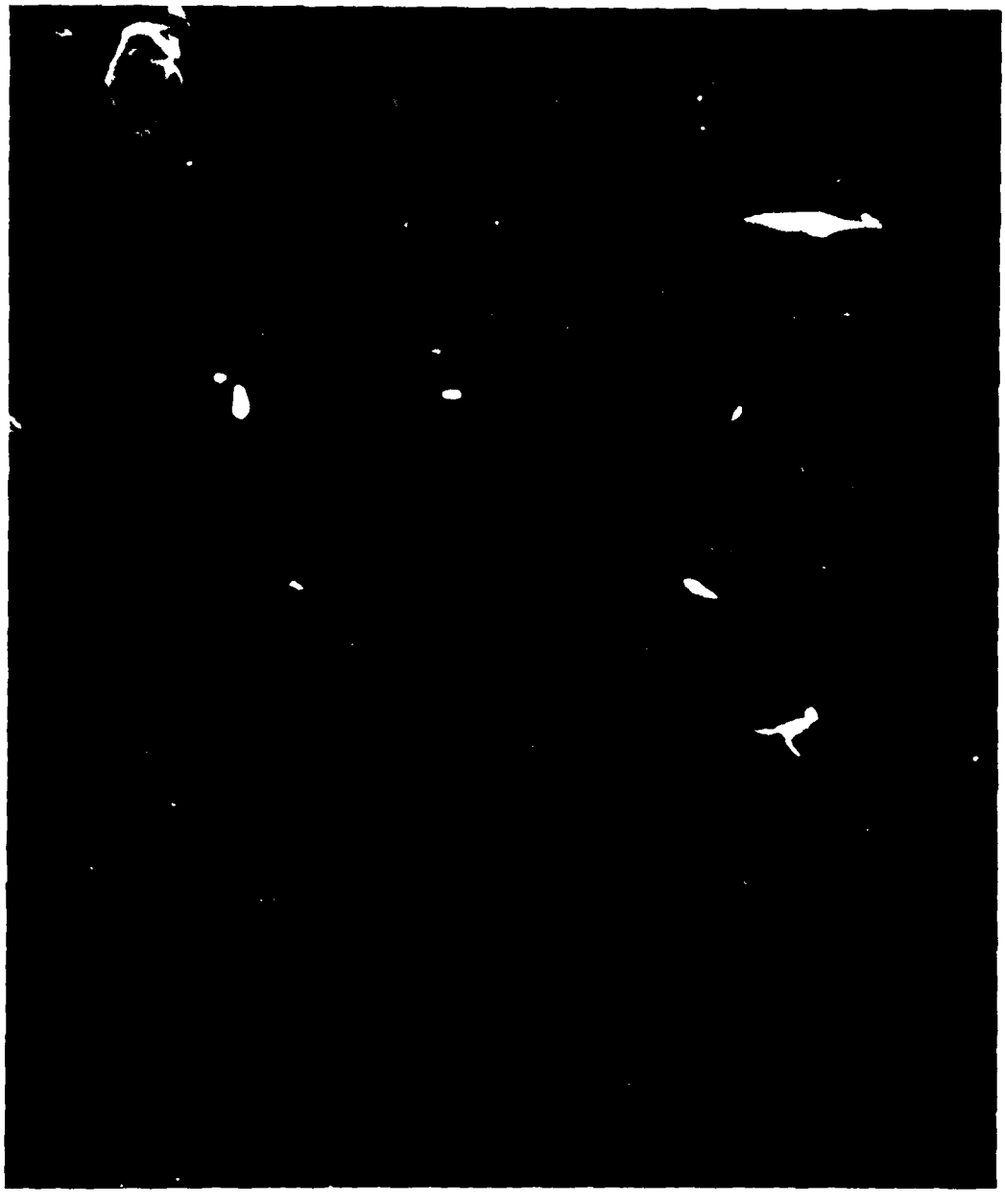
PHOTOGRAPHS OF INITIAL BACTERIAL ATTACHMENT

- PLATE (25) -

UNIMMERSED SLIDE SECTION (1000 X)
Showing dust and other inorganic materials.

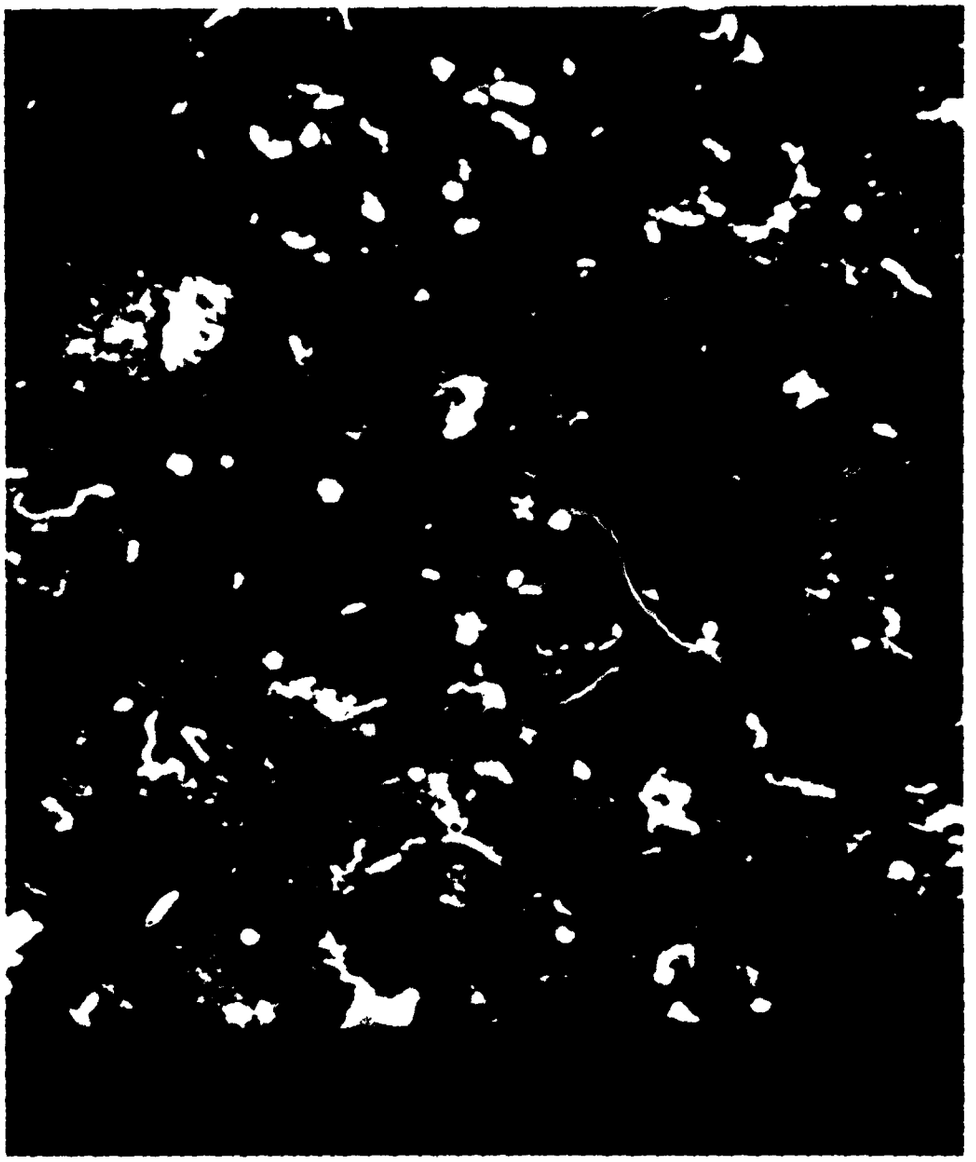
- PLATE (26) -

GLASS SLIDE SECTION IMMERSED 12 HOURS (1000 X)
Showing scattered coccus (round) and
Bacillus (rod-shaped) bacteria.



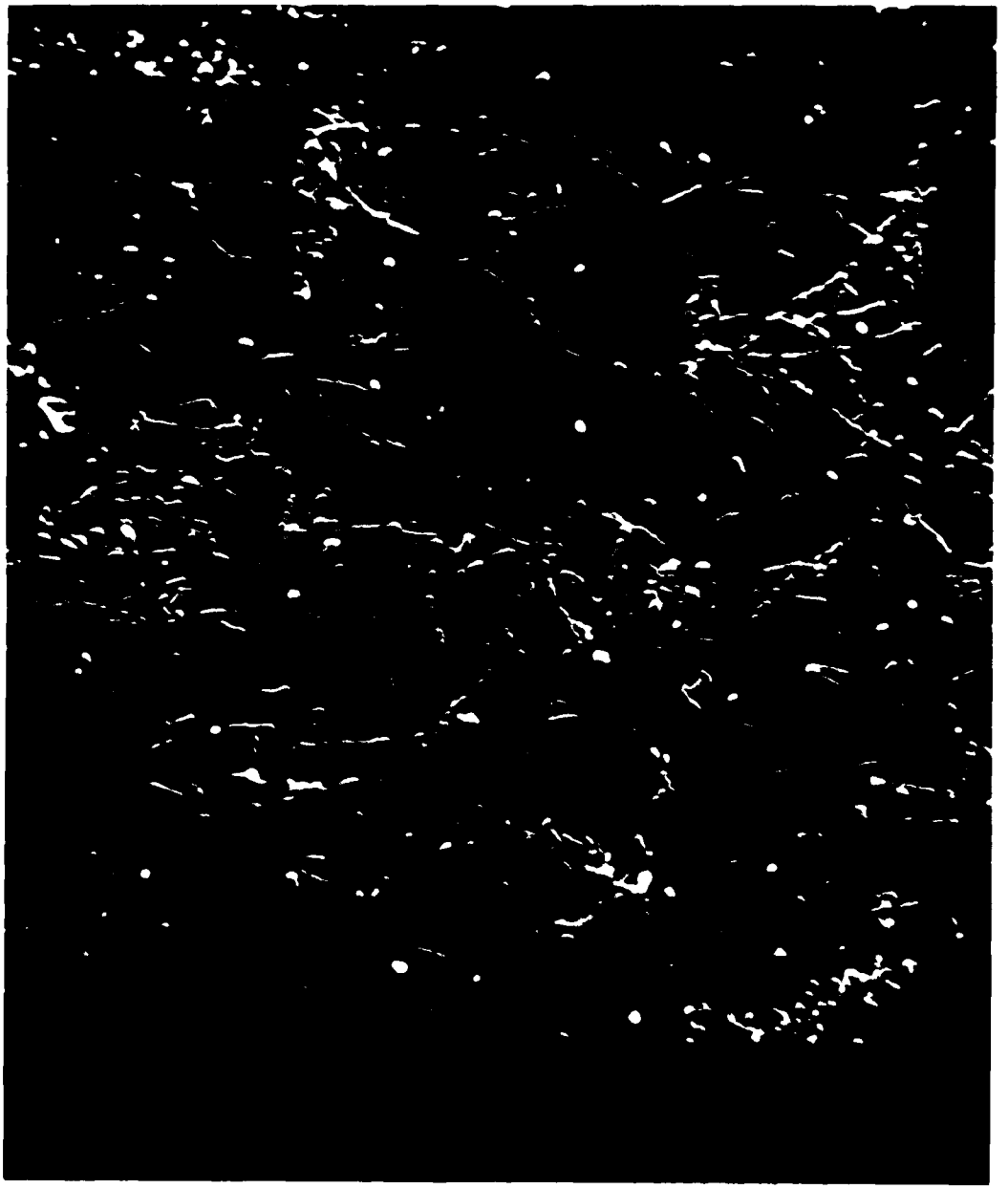
- PLATE (27) -

GLASS SLIDE SECTION IMMERSED 24 HOURS (1000 X)
Showing many different types of bacteria attaching
and growing on the substrate surface.



- PLATE (28) -

GLASS SLIDE SECTION IMMERSED 48 HOURS (1000 X)
Showing well established bacterial colonies nad chains.



- PLATE (29) -

DETAIL OF GLASS SLIDE SECTION IMMERSED 48 HOURS (1000 X)
Showing mucopolysaccharide "glue" surrounding bacteria.

- PLATE (30) -

GLASS SLIDE SECTION IMMERSED 72 HOURS (1000 X)
Showing extensive bacterial colonization
of the substrate surface.

- PLATE (31) -

GLASS SLIDE SECTION IMMERSED 96 HOURS (1000 X)
Showing many bacteria and a few "small sheep"
surrounded by mucopolysaccharide "glue"

- PLATE (32) -

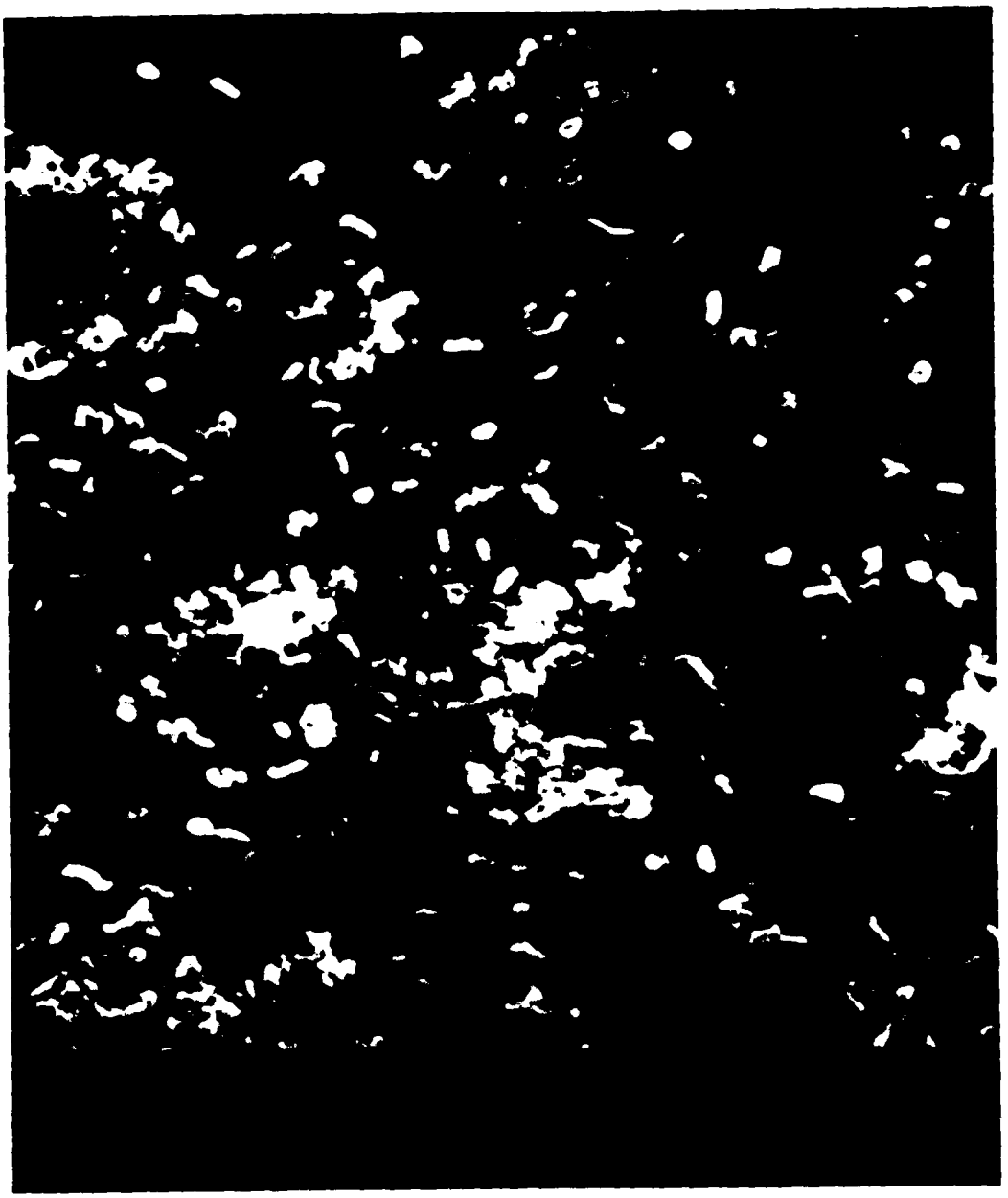
GLASS SLIDE SECTION IMMERSED 120 HOURS (1000 X)
Showing extensive build-up of existing bacterial colonies.

- PLATE (33) -

GLASS SLIDE SECTION IMMERSED 144 HOURS (1000 X)
Showing bacteria and bacterial colonies covering much of the substrate surface.

- PLATE (34) -

HYCAR SECTION IMMERSED 144 HOURS (1000 X)
Showing an extensive layer of organic material
and bacteria, but no "small sheep".



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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

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7. AUTHOR(s) Olson, Mark Carl		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS United States Naval Academy, Annapolis.		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS United States Naval Academy, Annapolis.		12. REPORT DATE [illegible]
		13. NUMBER OF PAGES [illegible]
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) [illegible]
		15a. DECLASSIFICATION DOWNGRADING SCHEDULE

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18. SUPPLEMENTARY NOTES

Accepted by the U. S. Trident Scholar Committee.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Marine fouling organisms

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Marine biological fouling is a complex, successive process which can severely impair the performance of marine vessels. This process is expensive to the Navy due to the time and money used in fouling prevention and compensation. In this analysis, a series of three interrelated experiments were conducted which analyzed the initial stages of marine microbiological fouling in the Severn River estuary. In the first experiment, glass microscope slides were exposed to natural fouling organisms at (OVER)

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"sampling stations" located along the length of the estuary. Slide and water samples were collected at set intervals, and a comparrison was made between the relative degree of fouling and individual water parameters experienced at each individual station. Results of the analysis showed low fouling to occur in waters which were relatively low in temperature and oxygen content, but high in salinity. The second experiment compared the surface energies of a set of substances to the degrees of fouling and organism attachment they demonstrated. Surface energies were determined using a contact goniometer, samples were exposed to natural fouling organisms in a controlled environment, and degrees of fouling and organism retention were determined using dissection and scanning electron (SEM) microscopes, and a hydrous bacterial sprayer (HBC). Results showed definite surface energy ranges which would experience low degrees of fouling or organism retention. In the third experiment, an artificial sea water solution was created and organically enriched in order to analyze solutions which would support life and promote the transfer of fouling bacteria. It was discovered that each of the nutrient solutions would support life for an extended period of time, while straight artificial sea water and distilled water would only allow bacterial transfer for a limited period of time. During each of the experiments common or significant fouling organisms were observed and noted. One such observation in experiment two, discovered extraordinary organisms, or organism remnants, referred to as "sheep", which were significant due to their strength of adhesion on substrates, and their composition of primarily metals.